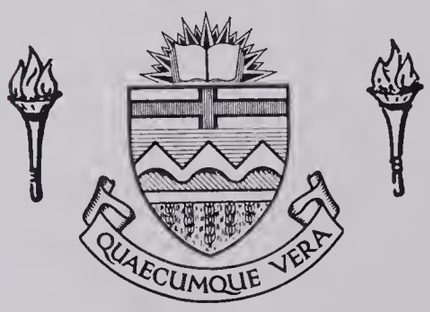

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LIPID METABOLISM IN BLATTELLA GERMANICA L.

by

Y. S. KRISHNAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies, a thesis entitled "Lipid metabolism in Blattella germanica L." submitted by Y.S. Krishnan in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Changes in the lipid composition of Blattella germanica L. during embryonic and post embryonic development were investigated by a combination of column, thin-layer and gas-liquid chromatography. During embryogenesis the loss of dry matter was mainly due to utilization of triglycerides for energy requirements. Hydrocarbon and sterol content increased slightly. Mono- and di-glyceride and free fatty acid content increased substantially. Fatty acid analysis revealed the presence of 17 fatty acids ranging in chain length from C_6 to C_{22}. Oleic acid was the most abundant. Palmitic acid was second in order of abundance. Phospholipid content increased during embryonic and post embryonic development. Lecithin, cephalin and sphingomyelin were the major phospholipids. During nymphal development all the lipid fractions increased approximately in proportion to the increase in body weight. Lipid composition of the nymphs and adults were similar.

Under non-aseptic conditions, fatty acids were not essential for completion of development. Addition of unsaturated 18 carbon fatty acids enhanced growth and increased the number of adults obtained. Omission of these unsaturated fatty acids from the diet resulted in a drastic decrease in linoleic and linolenic acid content of the depot fat. There was no correlation between susceptibility to malathion and the total lipid content or the degree of saturation of depot fatty acids.

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I. INTRODUCTION

The chemical composition of an organism provides valuable information on its biological activity. Lipids, one of the major body constituents in animals, are of high caloric value and their catabolism yields 2.2 and 1.6 times as much energy per gram as carbohydrate and protein respectively. They also yield about twice as much metabolic water and play an important role in terrestrial animals. Lipids include neutral fats, phospholipids, cerebrosides, sterols and fat soluble vitamins.

Many earlier works on insect lipids were restricted to gross quantitative analysis. The chemistry of insect fats has been reviewed by Timon-David (1930), Scoggin and Tauber (1950) and Hilditch (1956). In the past decade the volume of literature on the lipid composition of insects has increased tremendously as is evidenced by the publication of a number of reviews (Fast 1964, Gilby 1965, Kinsella 1966b, Gilbert 1967a). The remarkable growth of this field has been principally due to the development of better analytical techniques. The advent of thin-layer and gas-liquid chromatography in particular has enormously facilitated the purification and identification of complex lipids.

In insects energy is stored mainly in the form of lipids in a special organ, "the fat body". Much of the food eaten by insects during the immature stages is converted into fat and stored in the cells of the fat body. During embryonic development, neutral glycerides serve as the major energy substrate (Kinsella 1966b).

The chemical composition of an organism can be influenced by a number of factors like stage of development, physiological condition, starvation, type of food, environment,etc. Though fatty acids are not a dietary requirement for growth for most insects, they have been reported to influence the character of depot fat (Fast 1964). There has been a number of papers dealing with changes in quality and quantity of lipid induced by dietary means and increased tolerance to chlorinated hydrocarbon insecticides (Munson, Padilla and Weismann 1954).

Blattella germanica L. has long been employed as a subject of physiological and toxicological investigations. Lipid biochemistry in this insect, on the other hand, is one of the neglected areas.

The purpose of the present study was to investigate the qualitative and quantitative changes in the lipid composition of B. germanica during embryonic and post embryonic development. The effect of some dietary fatty acids on the lipid composition was examined. The effect of the dietary lipids on the susceptibility of this insect to malathion was also investigated.

II. CHANGES IN LIPID COMPOSITION DURING EMBRYONIC AND POST EMBRYONIC DEVELOPMENT

1. Introduction

During insect ontogeny important quantitative and qualitative changes may occur in the lipid fraction. Needham (1931) proposed a theory of a succession of energy sources during ontogeny; the embryo first utilizing carbohydrate primarily, then protein and finally fat. The literature on chemical composition of insect lipids has been reviewed by Timon-David (1930), Scoggin and Tauber (1950), Hilditch (1956), Niemierko (1959), Babcock and Rutschky (1961), Gilmour (1961), Kilby (1963), Fast (1964), Gilby (1965), Kinsella (1966b) and Gilbert (1967a).

Embryonic stages:-

A number of studies on lipid content of orthopteran eggs indicate that, in general, the lipid content ranges from 2.5 to 14% of the wet weight. The lipid concentration declines during development of the embryo. A large part of the loss appears to be in the triglyceride fraction; lipids are the major source of energy for the developing embryo. The greatest loss of lipids occurs during the later stages of development.

Probably the earliest work on the lipid content of an orthopteroid egg was that of Dubois (1893), who reported that newly laid eggs of the Algerian locust, Acridium peregrinum Oliv. contain 4 to 5% fatty material (wet weight basis) and this was considerably reduced during development. No quantitative estimate of the loss was given. Slifer (1930) reported that 9 to 12% of the wet weight of the newly laid eggs of Melanoplus differentialis (Thomas) was fatty acid. During embryogenesis 54% of the initial fatty

acid reserve was catabolized. Most of the loss occurred in the post diapause period. The iodine value remained constant during embryonic development. This was confirmed by Boell (1935) and Hill (1945). On the basis of respiratory studies, Boell (1935) calculated the loss of fatty acids as 67% of the initial store. Hill (1945) showed that carbohydrate formed the major energy source during the first 5 days of development. Protein and fat were chiefly used in the prediapause and diapause period. During post diapause fat catabolism accounted for 90% of the oxygen consumed.

Carausius (Dixippus) morosus (Br. and L.) used 26% of the lipid reserve during embryonic development and the fat content was reduced from 31% of the dry matter to 23% at the time of hatching (Lafon 1950).

Blackith and Howden (1961) and Allais et. al. (1964) recorded loss of lipids during embryogenesis in Locusta migratoria (L.). The latter authors observed that lipids accounted for 26% of the dry weight in the newly laid eggs (78.5% triglyceride, 19.5% phospholipids and 2% sterols) and showed a total decrease of 31.2% to form 20.7% at the end of embryonic development (triglycerides 66%, phospholipids 19.5% and sterols 3%). They concluded that this decline in lipid content was due only to catabolism of triglycerides. Phospholipids consisted chiefly of lecithins (70.5%) and cephalins (26.5%) and a small amount of sphingomyelin. The phospholipid content increased by 60% during embryogenesis, but no qualitative changes were observed. Sterol content remained constant and for the most part was in the free form.

Kinsella and Smyth (1966) and Kinsella (1966a,c,d) made an exhaustive study of the lipids of Periplaneta americana L. During embryo-

genesis, total extractable lipids decreased from 39.5% to 23.2% of the dry weight, mainly due to catabolism of the triglyceride fraction. There was an increase in the mono- and di-glyceride fractions (Kinsella and Smyth 1966). Sterol content remained constant during development. The sterol esters of newly extruded oothecae contained mainly palmitic, stearic, oleic and linoleic acids. Palmitic and stearic acid content decreased during development (Kinsella 1966d). Sphingomyelin, lecithin and cephalin are the major phospholipids. Small amounts of lysolecithin, phosphatidyl inositol and cerebroside were also found. Total phospholipid content increased fourfold during development. Sphingomyelin and cephalin content tripled and lecithin content doubled (Kinsella 1966a). There was close similarity in the fatty acid composition of the total lipid, neutral lipid and triglyceride fractions. Palmitic stearic, oleic and linoleic acids content accounted for 95% of the total fatty acids. The phospholipid fraction, however, had a greater amount of linolenic acid (Kinsella 1966c).

The lipid picture for the period of embryogenesis in Leucophaea maderae (Fab.) was characterized by a decreasing content of embryonic triglyceride and an increasing proportion of phospholipids (Gilbert 1967b).

Post embryonic stages:-

The lipid of post embryonic stages of orthopteroid insects vary over a wide range (1.7 to 16% of the wet weight). The immature stages, in general, have a higher lipid content.

Tsujimoto (1929) analyzed the fat of Oxya japonica (Fabr.). This species contained 3% fat (dry weight basis) and had a saponification number of 175, an iodine value of 122.6 and 15.7% unsaponifiable matter.

Palmitic, stearic, oleic and linoleic acids were identified. Seventy five per cent of the fatty acids were unsaturated. Body lipids of Acheta mitrata constituted 2.4% of the fresh weight. Unsaponifiable matter made up 11.3% of this and contained 45.5% sterols.

Sacharov (1930) reported that 3 to 5 day old nymphs of L. migratoria contained 2.8% fat. Matthee (1945) investigated some of the biochemical differences between the solitary and gregarious phases of L. migratoria and Locusta pardalina Walk. The fat content of L. migratoria solitaria adult was 11.0% of the dry weight and increased to 14.0% in L. migratoria gregaria. Similarly the fat content of the solitary phase adults of L. pardalina increased from 12.8 to 14.6% (dry basis) in the migratory phase. Fawzi, Osman and Schmidt (1961) recorded a much higher fat content in the migratory phase of L. migratoria; 10.4% of the wet weight in females and 14.6% of the wet weight in the males.

The lipid content of the German cockroach, B. germanica was studied by Mellampy and Maynard (1937). The lipid content of the nymphs, females with egg capsules and males was 5.7, 4.8 and 1.7% of the wet weight respectively. The Iodine Number was 69 for the nymphs and 74 for the females and males. In a later study McCay (1938) reported that of the dry weight of adults 15.6 to 17.1% was ether extractable material.

Lipid content of P. americana adults has been studied by a number of investigators. Schweet (1941) reported that the lipid content of adult females and males were 28.6 and 25.5% of the dry weight. According to Munson and Gottlieb (1953) the lipid content of the nymphs, adult males and females was 7.7%, 7.1% and 8.9% of the wet weight respectively. Siakotos

and Zoller (1960) and Kinsella and Smyth (1966) reported that 30% of the dry matter was lipids. Lofgren and Cutkomp (1956) recorded much lower values, 13.9% and 14.5% of the dry weight in females and males. Kinsella (1966a,c,d) compared the lipid composition of the nymphs and adults with those of the various stages of the embryo. The lipid composition of nymphs and adults were quite similar. Neutral lipid accounted for 75% of the total lipids. Lecithin, cephalin and sphingomyelin were the predominant phospholipids in that order of abundance (Kinsella 1966a). The fatty acid pattern of the various lipid fractions were similar to those found in the embryo (Kinsella 1966c).

On a wet weight basis, the adults of the grasshopper Melanoplus atlanis Riley consisted of 0.8% neutral fat and 2.4% fatty acids (Giral, Giral and Giral 1946). The free fatty acids consisted of stearic, palmitic and arachidic acids unsaturated C_{16}, C_{18}, C_{20} and C_{22} acids. Linolenic acid was not present but triethenoic acids of the C_{20}-C_{22} series appeared to be present. The polyunsaturated acids with more than 18 carbons were present in large amounts (46.2% of the total fat).

Components of the body fat of Taeniopoda auricornis Walk. was reported by Giral, Giral and Giral (1943). Fatty acids of the females consisted of 35% saturated acids, 6.5% oleic acid and 58.5% linoleic acid. Fatty acids of the males contained 15.5% saturated acids, 24.0% oleic and 60.5% linoleic acid. The lipids of the females contained 5.1% unsaponifiable matter; of the males 6.5%.

Giral (1946) found that the lipids extracted from the adults of Sphenarium purpurascens (Charp.) contained a very high proportion of free

fatty acids. Glycerides were present in very small amount. The fatty acids consisted of 22.1% saturated acids, 9.6% palmitoleic acid and 35.5% oleic acid. Of the fatty acids, 25.8% had a chain length of more than 18 carbons.

Barlow (1964) found that palmitic, oleic, linoleic and linolenic acids accounted for 95% of the fatty acids in the body fat of Melanoplus sanguinipes(Fab.)

II.2. Materials and methods:-

II.2.1. Laboratory rearing of the roaches:-

The roaches were fed rabbit pellets (3.5-4.5% lipids) and were kept in battery jars with pieces of folded paper which served as resting and hiding space. A one pound narrow mouthed jar was used as a water reservoir and a cotton wick was provided, the upper end being wrapped with absorbant cotton to make it fit tightly in the mouth of the bottle. Water and food was replenished once every three weeks. The roaches were raised at a temperature of 30 ± 1 C. Two to three hundred individuals were raised in one jar and adults were collected once every three days. Adults collected on the same day from different jars were pooled and used for lipid analysis after one week.

II.2.2 Extraction and purification of lipids:-

The procedure of Folch, Lees and Sloane-Stanley (1957) was followed. The insects were placed in a glass vial with 20 volumes of a chloroform : methanol mixture (2:1 v/v) and homogenized in a Potter-Elvehjem homogenizer for 30 minutes and filtered through a sintered glass funnel into a glass stoppered vial. The filtrate was shaken for 3 to 4 minutes with 0.2 volumes

of 0.90% sodium chloride solution. The mixture was centrifuged for 5 minutes at 400 g and the upper methanol:water:salt layer was removed with a fine pipette. To ensure complete removal of the solutes in the upper phase, the interphase was rinsed three times with a small volume chloroform:methanol:saline (3:47:48 v/v/v). The lower phase was evaporated to dryness in a rotary flash evaporator, dissolved in small volume of chloroform and stored at -10 C under nitrogen until further use.

II.2.3 Separation of lipid classes:-

The total lipid extract was placed on a 30 g silicic acid: Hyflo super cell column (2:1 w/w; column dimensions 2.2 x 14 cm) and eluted first with 200 ml of chloroform to obtain the neutral lipids. The phospholipids retained on the column were then eluted with 200 ml of chloroform : methanol (1:1 v/v) mixture. The eluates were then evaporated to dryness and weighed. The neutral lipid fraction was then placed on a 30 g deactivated florisil column (2.2 cm x 15 cm). The lipids were eluted according to the method of Carrol (1961). The solvents used and the various fractions obtained are presented in Table 1. The solvents of the isolated lipid classes were first removed in a flash point evaporator and were then placed in a vacuum oven at 40 C in tared planchets and the solvents removed. The planchets were reweighed to obtain the weights of the individual classes. The efficiency of the separation of these classes of compounds was checked by thin-layer chromatography (Mangold 1961).

II.2.4. Analysis of fatty acids by gas-liquid chromatography:-

Aliquots of lipid fractions were transmethylated by refluxing in 5 ml of 5% sulfuric acid in dry methanol (w/v) for 3 hours (Patton et al. 1964).

Table 1.

Solvent systems used in Florisil column chromatographic separation of neutral lipid fractions (method of Carrol 1961)

Eluting solvent	Volume in ml	Eluent
Hexane	50	Hydrocarbons
5% ether*in hexane	120	Sterol esters
15% ether in hexane	150	Triglycerides
25% ether in hexane	150	Sterols
50% ether in hexane	150	Diglycerides
2% methanol in ether	150	Monoglycerides
4% acetic acid in ether	150	Free fatty acids

* diethyl ether

An equal volume of distilled water was added and the methyl esters extracted three times with a small volume of redistilled petroleum ether (30-60 C). The combined ether extract was dried over anhydrous sodium sulfate and the petroleum ether removed under a stream of nitrogen. The methyl esters were then dissolved in a small volume of spectranalyzed n-hexane and small aliquots were used for separation by gas-liquid chromatography.

Qualitative and quantitative analyses of fatty acid methyl esters were made with a Beckman Model GC 5 Gas Chromatograph equipped with a dual hydrogen flame ionization detector. Copper columns (15' long, 1/8" O D) containing Chromosorb P (60-80 mesh) coated with DEGS (diethylene glycol succinate, 20% by weight of the solid support) were used. Since the column was continuously maintained at 200 C, the amount of liquid phase in time was reduced. This resulted in reduction of retention time. Standard methyl esters of fatty acids for comparative purposes and quantitation were obtained from Mann Research Laboratories. Unknown peaks were tentatively identified by logarithmic plot by the method of Hammerstrand (1966).

II. 2.5. Thin-layer chromatography of phospholipids:-

Phospholipid content was determined by silicic acid Hyflo super cell chromatography. The individual phospholipid classes were separated by thin-layer chromatography on 0.5 mm thick Silica gel G plates according to Wagner et. al. (1961). The developing solvent was chloroform : methanol : water (65:25:4 v/v). The developing chamber was lined with filter paper saturated with the solvent mixture. Following the chromatographic run, the thin-layer plates were air dried and exposed to iodine vapour for visualization of the phospholipids. Identification of the

individual phospholipid was accomplished by comparison of the R_f with those of pure standards obtained from Applied Science Laboratories, State College, U.S.A. and specific color reactions. Quantitation of the major phospholipids were based on the phosphorus content, determined colorimetrically according to the method of Bartlett (1959). Three determinations were made for each stage.

II. 3. RESULTS

II.3.1. Dry matter content:-

The changes in the wet weight, dry weight (obtained by drying at 100 C) and water content in the development of the ootheca and nymphs and the adults of the German cockroach are given in Table 12. The proportion of water in the egg was initially about 62%. This remained constant during the first five days of embryonic development and increased to about 75% by the 15th day. When calculated on a per egg basis, the wet weight of the egg increased from 1.276 mg to 1.617 mg, while the dry matter decreased from 0.486 mg to 0.399 mg (Fig. 1, Table 2). There was a slight drop in the % water content between the 1st and 3rd nymphal instars. In the subsequent nymphal instars and the adult water content remained more or less constant.

II.3.2. Total lipids:-

Table 2 reveals that the total lipid content of the eggs decreased during embryonic development (Figure 2). The newly formed egg, on an average, contained 0.178 mg of lipids which in 15 days of embryonic development decreased to 0.112 mg, representing a loss of 37% of the original lipid component. During the first 5 days of embryonic development,

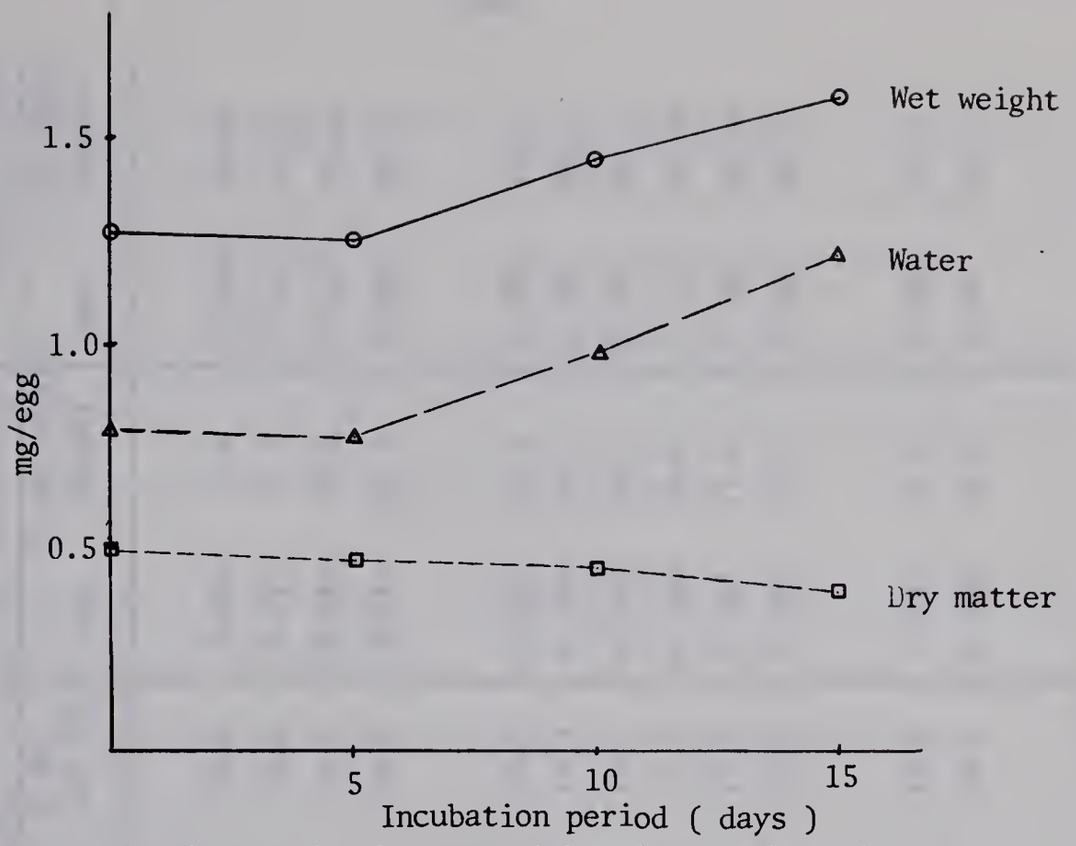


Figure 1 Changes in the wet weight, dry weight and water content of B.germanica during embryonic development.

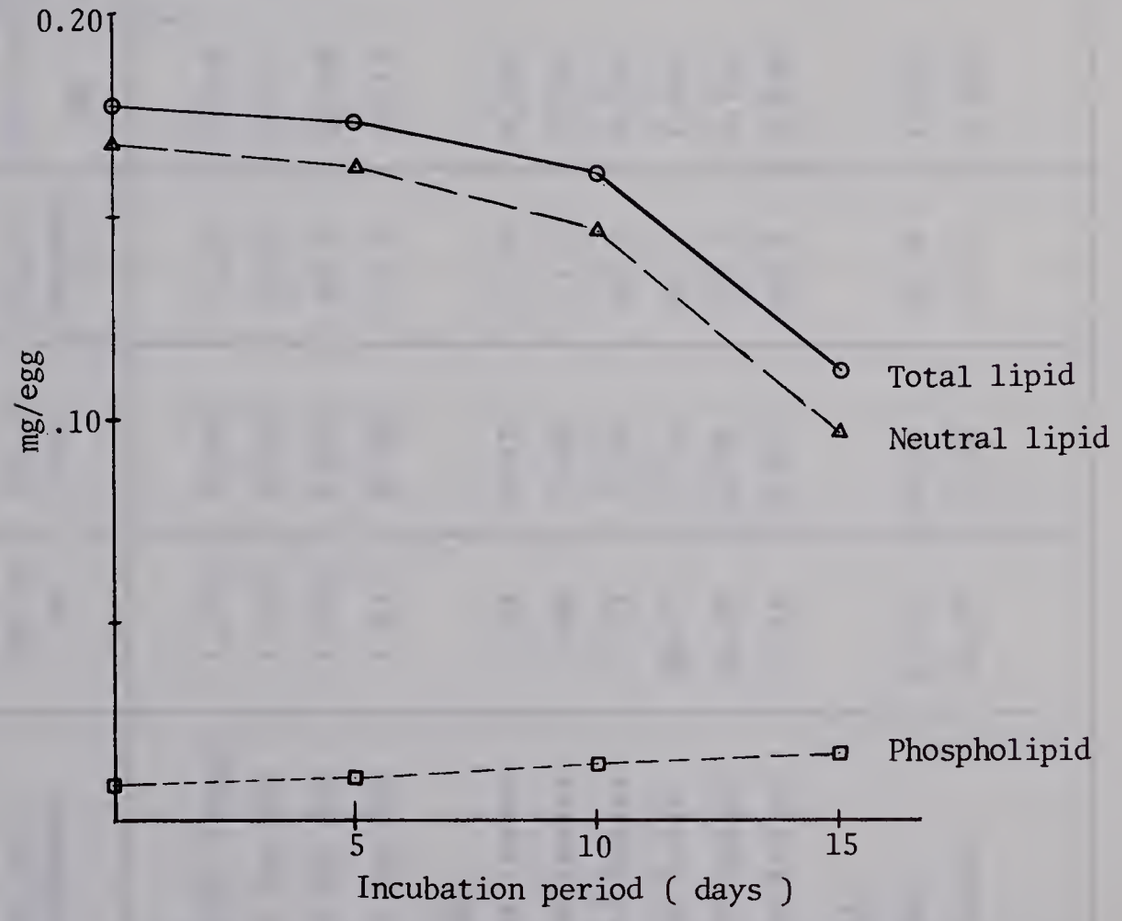


Figure 2 Changes in the total lipid, neutral lipid and phospholipid content during embryonic development of B.germanica.

Table 2

Changes in the dry weight, total lipid, phospholipid and neutral lipid content per individual during embryonic and post embryonic development of *B. germanica* L.

Stage of Development	Wet wt. mg	Dry wt. mg	Water Content %	Lipid Content			Phospholipid		Neutral lipid	
				mg.	% of wet wt.	% of dry wt.	mg.	% of total lipids	mg.	% of total lipids
Egg										
newly formed	1.276	0.486	61.9	0.178	14.0	36.6	0.010	5.4	0.168	94.6
5 day old	1.252	0.476	61.9	0.173	13.9	36.6	0.011	6.3	0.162	93.7
10 day old	1.442	0.453	68.5	0.160	11.1	35.3	0.014	8.6	0.146	91.4
15 day old	1.617	0.399	75.3	0.112	6.9	28.1	0.016	13.9	0.096	86.1
Nymph										
1st instar	2.25	0.62	72.6	0.123	5.5	19.8	0.021	17.3	0.102	82.7
2nd instar	5.09	1.49	71.3	0.279	5.5	18.7	0.054	19.7	0.225	80.3
3rd instar	9.92	2.99	69.9	0.570	5.8	19.1	0.095	16.7	0.475	83.3
4th instar	20.47	6.51	68.2	1.402	6.9	21.5	0.230	16.4	1.172	83.6
5th instar	36.85	11.59	68.5	2.141	5.8	18.5	0.363	17.4	1.768	82.6
6th instar	53.48	16.93	68.5	3.920	7.3	23.2	0.758	19.3	3.162	80.7
Adult										
Male	47.72	14.54	67.4	1.933	4.1	12.4	0.343	17.8	1.590	82.2
Female	64.78	19.79	69.4	3.699	5.7	28.9	0.658	17.8	3.041	82.2

lipid catabolism accounted for 50% of the loss of dry matter; between the 5th and 10th day 56% of the loss of dry matter was due to lipid loss; nearly all of the dry matter loss between the 10th and 15th day of incubation was due to lipid utilization. This indicates that during the early part of embryonic development, some other component(s) must have been used for fulfilling the energy requirements.

There was a progressive increase in the amount of lipids per individual insect as the development of the nymphs proceed, reaching a maximum in the 6th instar (Table 2). However, the lipid content dropped considerably in the adult stage. The females had twice as much lipids as the males.

II.3.3. Lipid class spectrum:-

At the beginning of embryonic development the neutral lipid constituted 94.6% of the total lipid, but by the time the eggs were 15 days old, it was lowered to 86% of the total lipid content (Table 2). Embryonic development resulted in a 43% loss of the neutral lipid reserve. During nymphal growth the neutral lipid content per individual increased more or less proportionally to the increase in body weight, reaching a maximum in the 6th instar. The adults have a lower neutral lipid content than the last instar nymphs.

The neutral lipid was fractionated into the following classes : hydrocarbons, free sterols, sterol esters, triglycerides, diglycerides, monoglycerides and free fatty acids. Table 13, in the appendix, is a summary of the analyses conducted at the various stages of development. The values are based on three analysis for each stage. This table clearly

Table 3

Lipid class spectrum of the neutral lipid fraction during various stages of B. germanica

	Hydro-carbons mg	Sterol esters mg	Sterols (Free) mg	Total sterols mg	Tri-glycerides mg	Di-glycerides mg	Mono-glycerides mg	Total glycerides mg	Free fatty acids mg
ootheca [1]									
0 days	.059	.045	.126	.171	4.775	.012	.028	4.815	.016
5 days	.062	.051	.120	.171	4.481	.049	.074	4.604	.042
10 days	.071	.063	.095	.158	3.876	.080	.101	4.057	.093
15 days	.074	.093	.101	.194	2.195	.124	.163	2.482	.143
Nymphs [2]									
1st instar	.006	.006	.005	.011	0.072	.005	.004	0.081	.004
2nd instar	.013	.012	.013	.025	0.160	.010	.009	0.179	.008
3rd instar	.025	.027	.028	.055	0.333	.027	.021	0.381	.014
4th instar	.058	.071	.068	.139	0.814	.067	.058	0.939	.036
5th instar	.139	.102	.090	.192	1.233	.050	.082	1.365	.072
6th instar	.164	.139	.234	.373	2.210	.143	.151	2.504	.121
Adults [2]									
Males	.100	.091	.112	.203	1.061	.079	.078	1.218	.069
Females	.195	.195	.136	.331	2.188	.134	.118	2.440	.075

1 weight in mg per ootheca 2 Weight in mg per individual

shows that the neutral lipid fraction consisted predominantly of triglycerides in all the stages. During embryonic development the level of triglyceride fell from 94% to 76% of the neutral lipid fraction. Gravimetrically there was a 54% loss of the triglyceride content. The loss in the triglyceride content (2.58 mg) was greater than the loss of dry matter (2.51 mg) and neutral lipid (2.16 mg). The proportion of mono- and di-glyceride as well as the free fatty acid content increased considerably during incubation. Quantitatively (Table 3) the mono- and di-glyceride content increased 6 and 10 times of the initial concentration per individual respectively. The free fatty acid content increased 7 fold.

The relative proportion of the various fractions remained more or less constant in all the nymphal stages (Table 14). Quantitatively, all the fractions increased in each successive nymphal instar (Table 3). Adult females had a neutral lipid class spectrum similar to the nymphs. Adult males had a slightly lower triglyceride level compared to other post embryonic stages.

Sterol content remained constant during the first 5 days of embryonic development. There was a drop between the 5th and 10th day but an increase to a higher amount by the 15th day (Table 3). The proportion of esterified sterol progressively increased from 26% in the newly formed oothecae to 48% in the 15 day old oothecae. Total sterol content increased in successive nymphal instars. The proportion of free and esterified sterol fluctuated. Sterol of females were predominantly in the esterified form while in the males the free forms predominated.

Hydrocarbon content increased gradually at successive developmental stages of the embryo. During nymphal development, the increase in the

hydrocarbon content more or less parallelled the increase in the total lipid content.

II.3.4. Phospholipids:-

The total phospholipid present in each stage is shown in Table 2. The % phosphorus content of the major phospholipid fractions (lecithins, cephalins and sphingomyelin) at various stages of development are recorded in Table 4. From this the amount of the major phospholipid fractions (in terms of phosphorus content) were calculated and shown in Table 4.

During the embryonic development total phospholipid content as well as the three major fractions increased. The proportion of phospholipid at the beginning of embryonic development averaged 6% of the total lipid content. In the 15 days of embryonic development it rose to 13% of the total lipid. Gravimetrically the total phospholipid content increased one and a half times. This indicates a synthesis of phospholipids. Once the nymphs had started feeding, the increment in the phospholipid content was in proportion to the increase in total fat content. During the six subsequent nymphal instars the level of phospholipids remained nearly constant at about 18% of the total lipids.

Phosphatidyl choline (Lecithin) and phosphatidyl ethanolamine (Cephalin) were the principal phospholipids. In addition to the above two and sphingomyelin, which were quantitated, phosphatidyl serine, phosphatidyl inositol, lysolecithin and phosphatidic acid were detected in trace amounts in all stages studied. During embryonic development the lecithin/cephalin ratio was lowered. Gravimetrically, however, lecithin content increased by a larger amount than cephalin. The relative proportion of the three major fractions remained

Table 4

Changes in the distribution of lipid phosphorus and phospholipid fractions during embryonic and post embryonic development of B. germanica

Stage	Lipid Phosphorus content		Distribution of Phosphorus in major fractions							Lecithin/cephalin ratio
			% distribution				µg/insect			
	%	µg/insect	Sphingo-myelin	Lecithin	Cephalin	Misc.	Sphingo-myelin	Lecithin	Cephalin	
Egg										
0 day	3.68	0.368	5.5	60.0	31.7	2.8	0.020	0.221	0.117	1.89
5 day	3.75	0.413	5.6	59.1	33.7	1.6	0.023	0.244	0.139	1.75
10 day	3.74	0.524	5.8	58.6	34.2	1.4	0.030	0.307	0.179	1.71
15 day	3.88	0.621	5.7	57.4	34.3	2.6	0.035	0.356	0.213	1.67
Nymphs										
Instar 1	3.97	0.834	5.7	56.4	34.7	3.2	0.048	0.470	0.289	1.63
Instar 2	3.90	2.106	5.8	56.4	33.7	4.1	0.122	1.188	0.710	1.67
Instar 3	3.89	3.696	6.2	56.6	32.9	4.3	0.229	2.092	1.216	1.72
Instar 4	4.00	9.200	5.5	58.0	32.7	3.8	0.506	5.336	3.008	1.77
Instar 5	3.78	13.731	5.7	55.8	33.8	4.7	0.782	7.656	4.638	1.65
Instar 6	4.02	30.472	5.7	56.9	33.4	4.0	1.737	17.339	10.178	1.70
Adults										
Male	3.98	13.651	5.7	56.6	33.9	3.8	0.773	7.676	4.628	1.67
Female	4.00	26.320	5.5	56.5	33.9	4.1	1.448	14.871	8.922	1.67

constant in the nymphal stages. Based on lipid phosphorus, the % composition of lecithin, cephalin and sphingomyelin were 6, 57 and 33% respectively. The lecithin/cephalin ratio varied from 1.63 to 1.67. The phospholipid composition of males and females were similar.

II.3.5. Fatty acid composition:-

Table 5 presents a summary of the gas chromatographic analysis of the fatty acids in the total lipid extract at 12 different stages of development. Figure 3a and 3b shows analysis of authentic standards and figure 4 is an analysis of an adult female. This analysis revealed the presence of 17 fatty acids ranging in a carbon chain length from 6 to 22. Of these $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were quantitated. It should be pointed out here that $C_{18:3}$ and $C_{20:0}$ could not be separated with the column used.

Oleic acid ($C_{18:1}$) was the largest component in all the stages and accounted for 42 to 48% of the total fatty acid fraction (Table 5). Palmitic acid ($C_{16:0}$) was next in order abundance. About 70% of the fatty acids were unsaturated. Besides oleic acid, linoleic acid ($C_{18:2}$) was present in fair amount (16 to 19%). Of the remaining 30% saturated fatty acids, palmitic acid ($C_{16:0}$) was the most abundant (25 to 30%).

The proportion of myristic acid ($C_{14:0}$) in the nymphs and adults was three times as much as in the embryonic stages. Palmitic acid content in the 0 and 5 day old oothecae were quite similar but it dropped to a lower % in the 10 and 15 day old oothecae. The reverse was the case with oleic acid. The % of the remaining major fatty acids were quite similar in all the fatty acid composition between sexes. During nymphal growth,

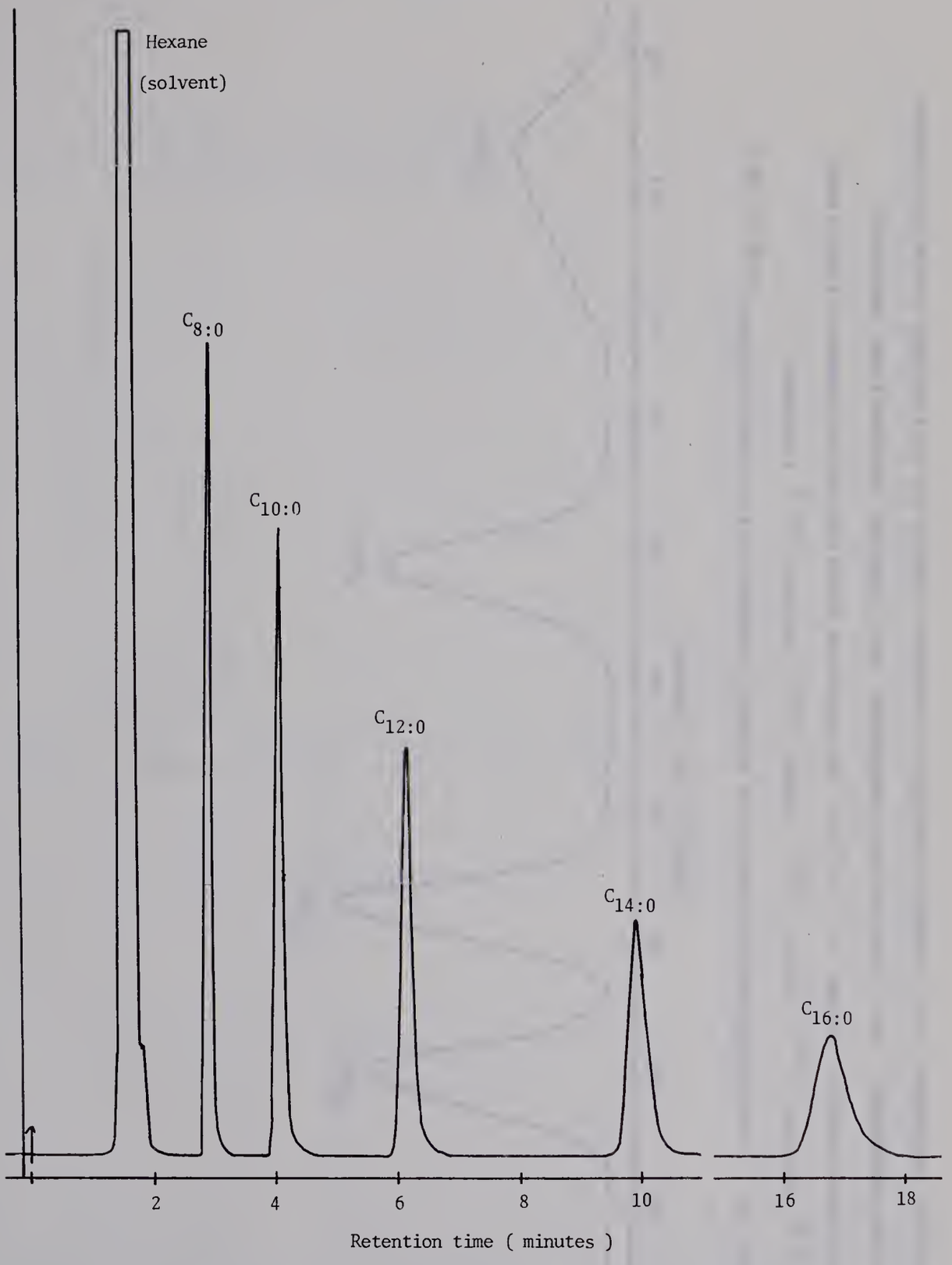


Figure 3a GAS CHROMATOGRAPHIC SEPARATION OF AUTHENTIC FATTY ACID METHYL ESTERS $C_{8:0}$ to $C_{16:0}$

$C_{8:0}$ Caprylate; $C_{10:0}$ Caprate; $C_{12:0}$ Laurate; $C_{14:0}$ Myristate; $C_{16;0}$ Palmitate.

Instrument settings: COLUMN, 15' x 1/8" OD., 20% Diethylene glycol succinate on chromosorb P 60/80 mesh; COLUMN TEMPERATURE 200 C; INJECTION PORT TEMPERATURE, 240 C; DETECTOR, Hydrogen flame ionization; DETECTOR TEMPERATURE, 240 C; HYDROGEN FLOW RATE, 25 ml per minute; CARRIER GAS AND FLOW RATE , Helium 40 ml per minute.

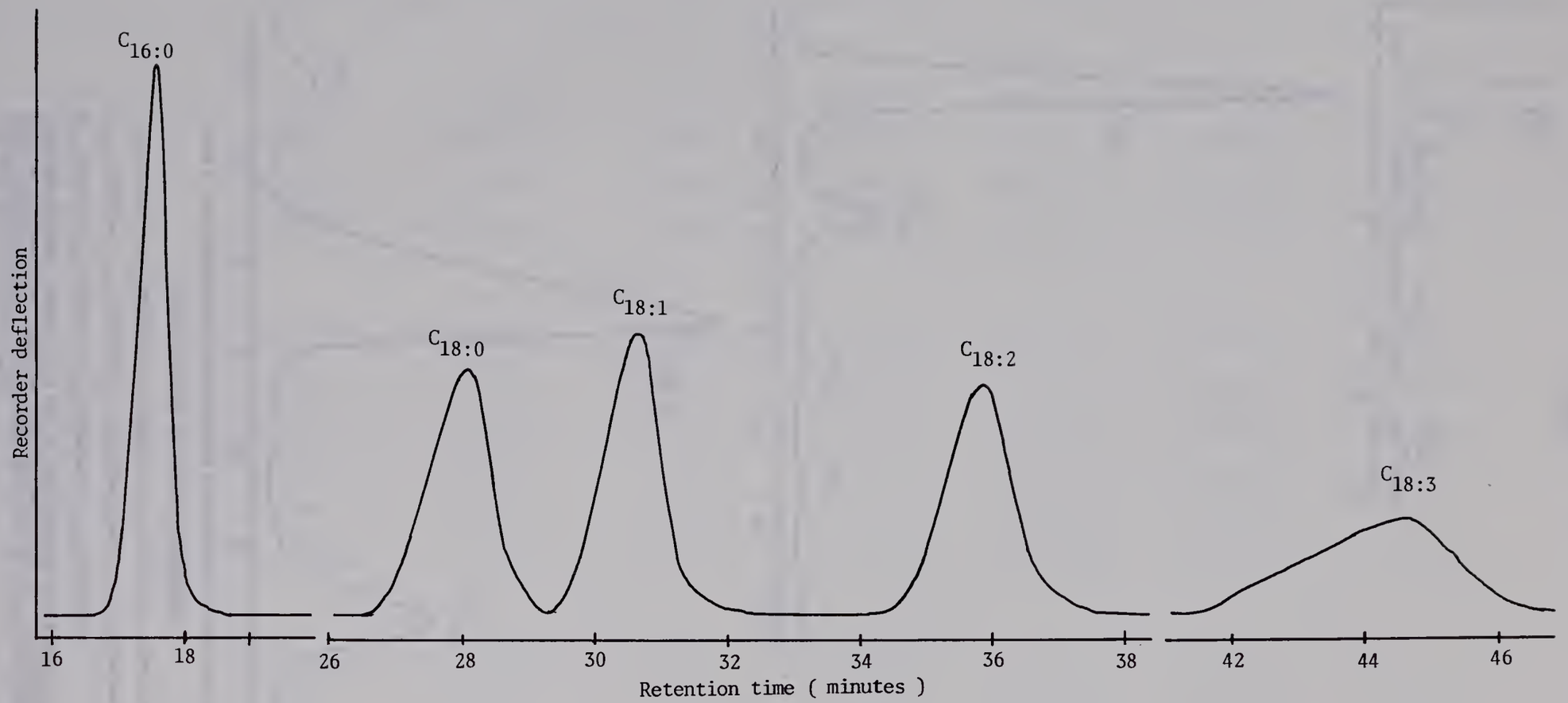


Figure 3b GAS CHROMATOGRAPHIC SEPARATION OF AUTHENTIC STANDARD FATTY ACID METHYL ESTERS $C_{16:0}$ to $C_{18:3}$

$C_{16:0}$ Palmitate; $C_{18:0}$ Stearate; $C_{18:1}$ Oleate; $C_{18:2}$ Linoleate; $C_{18:3}$ Linolenate.

Instrument settings : COLUMN, 15' x $^1/_8$" OD., 20% Diethylene glycol succinate on chromosorb P 60/80 mesh; COLUMN TEMPERATURE, 200 C; INJECTION PORT TEMPERATURE, 240 C; DETECTOR, Hydrogen flame ionization; DETECTOR TEMPERATURE, 240 C, HYDROGEN FLOW RATE, 25 ml/minute; CARRIER GAS AND FLOW RATE, Helium 40 ml/minute.

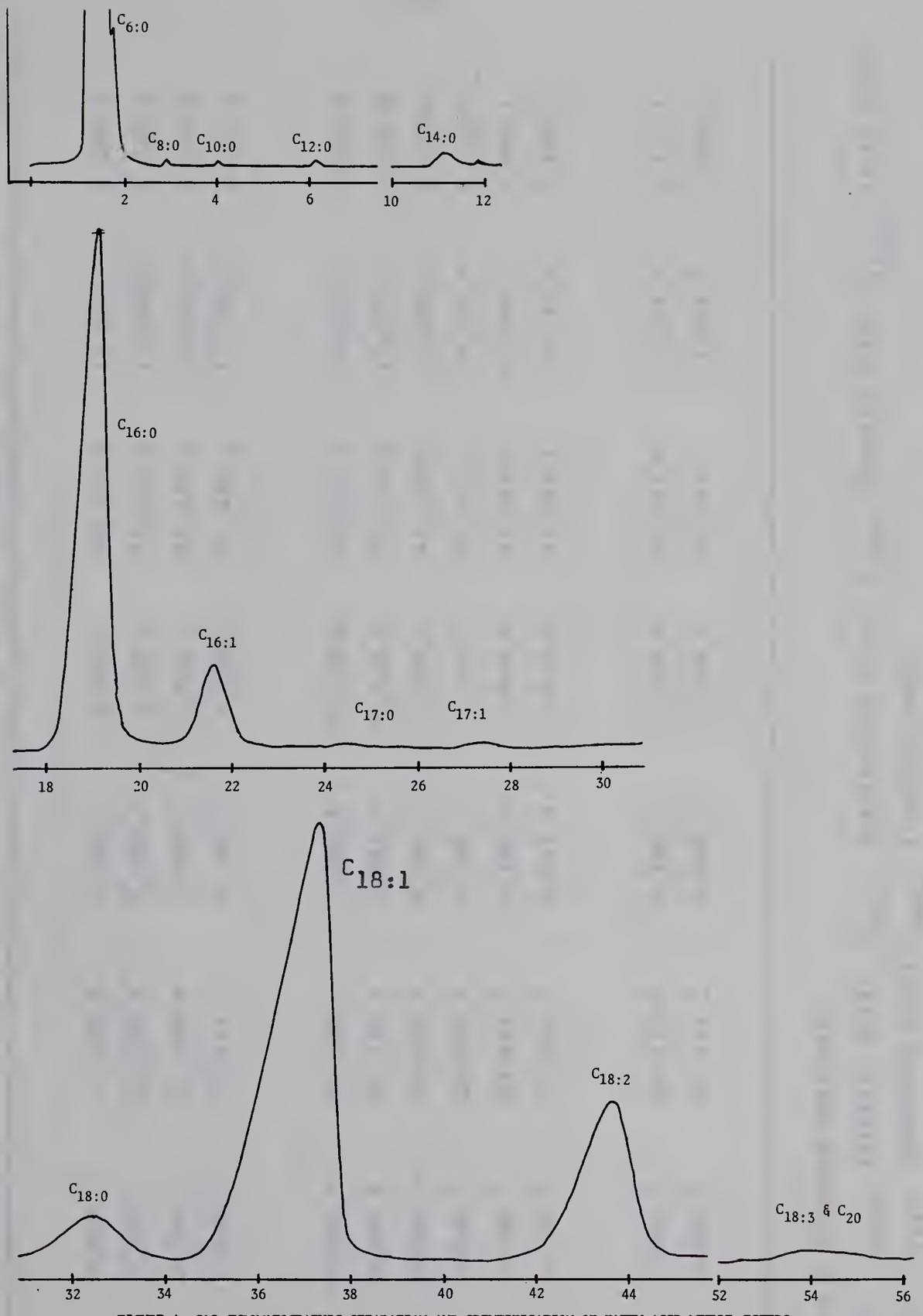


FIGURE 4 GAS CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF FATTY ACID METHYL ESTERS OF TOTAL LIPID OF ADULT FEMALE B.GERMANICA.

$C_{6:0}$ Caproate; $C_{8:0}$ Caprylate; $C_{10:0}$ Caprate; $C_{12:0}$ Laurate; $C_{14:0}$ Myristate; $C_{14:1}$ Myristoleate; $C_{16:0}$ Palmitate; $C_{16:1}$ Palmitoleate; $C_{17:0}$Heptadecanoate; $C_{17:1}$Heptadecenoate; $C_{18:0}$ Stearate; $C_{L8:1}$ Oleate; $C_{18:2}$ Linoleate; $C_{18:3}$ Linolenate; $C_{20:0}$ Arachidate.

INSTRUMENT SETTINGS: COLUMN, 15' x 1/8" OD, 20% Diethylene glycol succinate on Chromosorb P DETECTOR, Hydrogen flame ionization; DETECTOR TEMPERATURE, 240 C; INJECTION PORT TEMPERATURE, 230 C; COLUMN TEMPERATURE, 200 C; Hydrogen flow rate, 25 ml per minute; CARRIER GAS AND FLOW RATE, Helium 40 ml per minute.

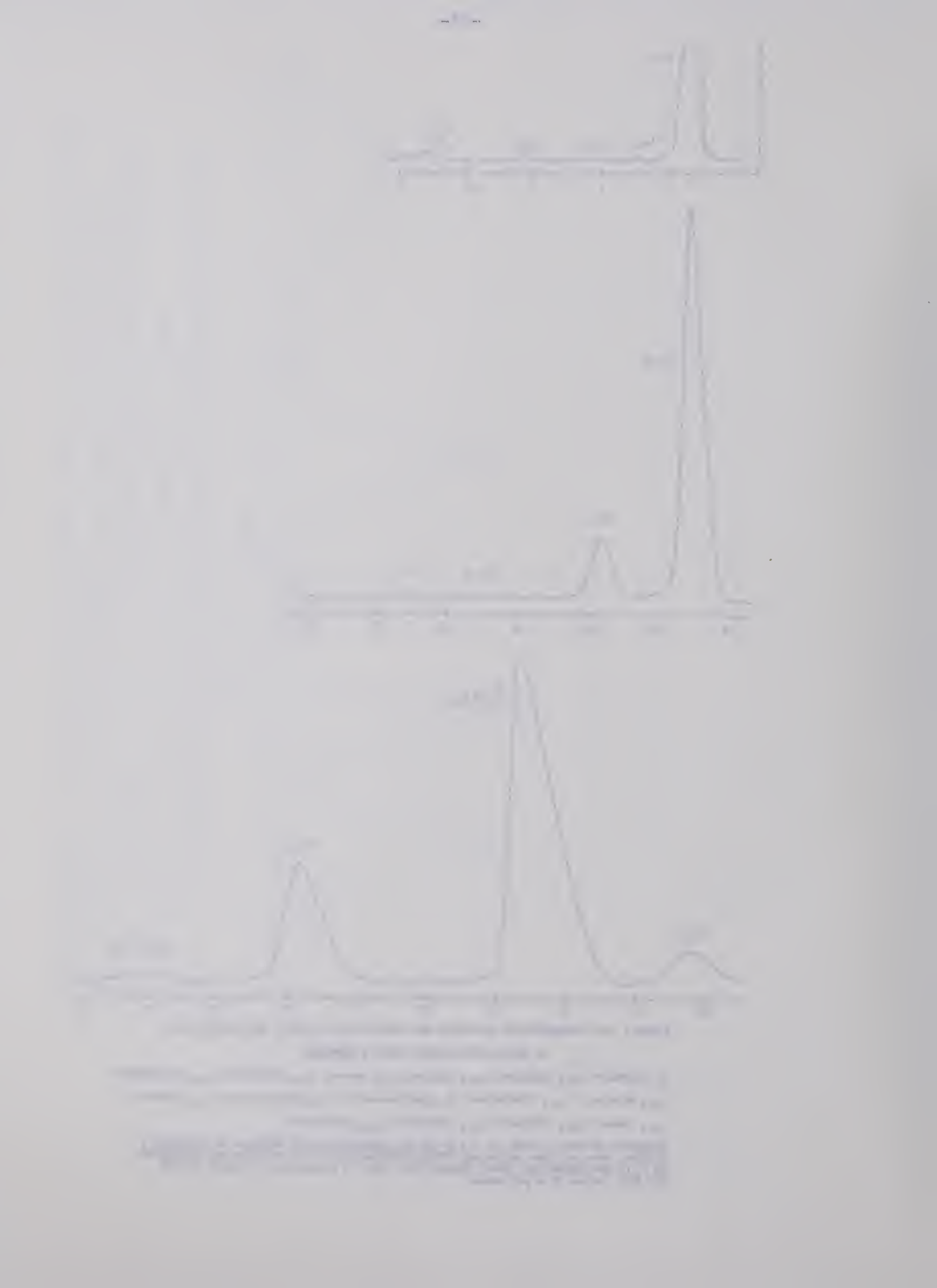


Table 5

Fatty acid composition of the total lipid extract of B. germanica L. at various stages of the life cycle

Stage	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ & $C_{20:0}$
Ootheca							
Newly formed	0.4±0.1*	29.1±0.9	4.2±0.3	2.3±0.3	44.2±1.9	17.6±2.7	2.2±0.3
5 day old	0.3±0.1	30.7±0.8	4.6±0.3	2.1±0.1	45.5±0.3	15.3±0.4	1.5±0.3
10 day old	0.3±0.1	25.6±0.9	3.6±0.2	3.6±1.1	48.3±0.1	16.5±0.3	2.1±0.9
15 day old	0.4±0.1	25.1±1.3	4.2±0.2	3.3±0.2	47.4±0.7	17.7±0.2	2.0±0.6
Nymph							
1st instar	0.9±0.1	24.2±0.7	3.4±0.4	4.7±0.6	45.5±1.2	19.2±3.1	2.1±0.7
2nd instar	0.9±0.1	26.1±2.1	3.8±1.0	4.4±0.5	44.7±2.2	17.6±0.5	2.5±0.6
3rd instar	1.0±0.0	28.8±0.4	4.9±0.2	3.6±0.1	42.2±0.4	17.0±0.5	2.5±0.6
4th instar	1.0±0.1	28.2±0.6	5.2±0.2	3.7±0.7	43.5±2.5	16.5±1.9	1.9±0.5
5th instar	1.2±0.2	29.4±1.3	6.1±0.6	3.4±0.4	42.2±1.8	15.7±0.7	1.9±0.1
6th instar	1.1±0.1	27.9±2.5	4.8±1.8	3.6±0.8	43.8±1.1	16.7±2.4	2.1±0.3
Adult							
Male	0.9±0.1	24.7±1.0	4.1±0.3	3.5±0.4	45.9±1.6	18.2±1.9	2.7±1.1
Female	0.8±0.1	27.1±1.2	4.6±0.3	3.3±0.1	45.4±1.2	17.0±1.1	1.8±0.3

*Mean of three samples ± standard deviation

$C_{14:0}$ Myristic acid; $C_{16:0}$ Palmitic acid; $C_{16:1}$ Palmitoleic acid; $C_{18:0}$ Stearic acid; $C_{18:1}$ oleic acid
$C_{18:2}$ Linoleic acid; $C_{18:3}$ Linolenic acid; $C_{20:0}$ Arachidic acid.

Table 6

Percent fatty acid composition of the total lipid, and isolated neutral and phospholipid fractions of B. germanica

Fatty acid	Stage: 4th instar nymph TL	NL	PL	Adult males TL	NL	PL	Adult females TL	NL	PL
$C_{14:0}$	1.0	1.3	0.6	0.9	0.7	0.5	0.8	1.0	0.4
$C_{16:0}$	28.2	30.6	14.5	24.7	28.6	18.3	27.1	31.8	15.0
$C_{16:1}$	5.2	5.6	3.1	4.1	3.8	3.7	4.6	4.5	2.9
$C_{18:0}$	3.7	3.7	6.1	3.5	3.4	3.7	3.3	2.7	4.2
$C_{18:1}$	43.5	41.4	43.3	45.9	45.0	44.9	45.4	44.0	45.9
$C_{18:2}$	16.5	15.3	30.2	18.2	16.4	25.8	17.0	14.3	27.3
$C_{18:3}$ & $C_{20:0}$	1.9	2.1	2.2	2.7	2.1	3.1	1.8	1.7	4.3

TL - Total lipid NL - Neutral lipid PL - Phospholipid

$C_{14:0}$ Myristic acid; $C_{16:0}$ Palmitic acid; $C_{16:1}$ Palmitoleic acid; $C_{18:0}$ Stearic acid; $C_{18:1}$ Oleic acid; $C_{18:2}$ Linoleic acid; $C_{18:3}$ Linolenic acid; $C_{20:0}$ Arachidic acid.

the proportion of palmitic and palmitoleic acid increased, but the proportion of linoleic and linolenic acid decreased.

Fatty acids contained in the isolated neutral and phospholipid fractions of the 4th instar nymphs, adult males and females were also separated by gas-liquid chromatography. Qualitatively there was no difference between the three types of lipids. Only 8 fatty acids were quantitated. Table 6 shows the relative proportion of these fatty acids. A comparison of the data shows that the proportion of these fatty acids in the neutral lipid fraction approximated the composition in the total lipid extract in all the 3 stages studied. Fatty acid composition of phospholipid fraction showed some differences. The proportion of myristic, palmitic and palmitoleic acids was reduced to half the relative proportion of these acids in total and neutral lipid fractions. The proportion of linoleic acid, on the other hand, was doubled. The phospholipid also contained slightly higher proportion of linolenic and arachidic acids. The fatty acids of the neutral lipid fraction had a higher proportion of saturated fatty acids (40%) than phospholipids (28%).

II.4. Discussion

Data presented indicate a progressive increase in the wet weight and a decrease in the dry matter content during embryogenesis. The changes reported here agree, in general, with those of Roth and Willis (1955a, 1955b). Ross (1929) and Parker and Campbell (1940) suggested that the wall of the ootheca of B. germanica in contact with the female's genital pouch may be permeable to water. Roth and Willis (1955b) demonstrated a difference in permeability between the anterior and posterior end of the ootheca of B. germanica. The anterior end, held by the female is lighter in colour and less sclerotized, was more permeable. They concluded that the increase in wet weight was due to absorption of water from the female. Similar phenomenon has been observed in Blattella vaga Hebard (Roth and Willis, 1955b) and Diploptera dytiscoides (Serv.) (Roth and Willis, 1955c).

The loss of dry matter was accompanied by a loss in the total lipid content. On the average, 75% of the loss of dry matter was accounted for by lipid catabolism. Many workers who studied the embryonic period have found that fat forms the main source of energy of the developing embryo (Tichimirov, 1885; Rudolfs, 1926; Fink, 1925; Slifer, 1930; Busnell, 1937; Lafon, 1950; Rainey, 1950; Rothstein, 1952; Gilbert and Schneidermann, 1961; Kinsella and Smyth, 1966; Gilbert, 1967b). The only exception was Tenebrio molitor L. which utilized glycogen for energy requirements (Ludwig and Ramazzotto, 1965). The loss in the initial lipid supply was comparable to the general average of 55% reported by Needham (1931) for various terrestrial animals.

A comparison of the neutral lipid content of newly extruded ootheca and 15 day old ootheca shows a 43% reduction of the initial supply.

Of the various lipid classes of the neutral lipids, only triglyceride shows considerable reduction. There is an increase in the mono- and diglyceride content. These findings agree with those of Slifer (1930), who showed that 54% of the neutral lipids are catabolized by M. differentialis during embryonic development. Tichimirov (1885) observed that neutral glyceride fatty acids diminished by 46%. Malacosoma americana (Fab.) utilized 87% of neutral lipids during incubation (Rudolfs1926). Gilbert and Schneiderman reported that the moth Hyalophora cecropia (L.) catabolized 57% of the initial neutral lipids during incubation. The Japanese beetle, Popillia japonica Newman loses 58% of the initial neutral lipid content during incubation. Allais et al.(1964) showed 53% loss of neutral lipid moiety. Kinsella and Smyth (1965) and Gilbert (1967) observed 55% loss of neutral lipids during embryogenesis in P. americana and L. maderae.

In the present work it has been shown that the hydrocarbon and sterol content increased only slightly. With the development of the embryo, the proportion of the esterified sterols increases. Tichimirov (1885), Allais et al.(1964) and Gilbert (1967b) reported similar findings during embryonic development of Bombyx mori (L.), L. migratoria and L. maderae respectively, whereas Rudolfs (1926) reported that the cholesterol in the eggs of M. americana disappeared by the time of hatching. Kinsella (1966d) and Gilbert (1967b) also observed an increase in the proportion of esterified sterols during incubation. The more or less constant sterol content ties well with the theory that insects lack the ability to synthesize sterols. However, it is rather difficult to correlate it with the known function of sterols, being a major constituent of cellular membranes. During morphogenesis, it would be expected to increase.

During nymphal growth and in the adults the sterol content increases in proportion to the increase with the total fat content. It has been shown that some dietary sterols can be converted into cholesterol (Gilmour 1961).

The fatty acid composition of B. germanica closely resembles that of P. americana (Kinsella 1966c). The predominance of oleic acid and palmitic acid is in conformity with other insects (Fast 1964). Only aphids and coccids are peculiar in having a large proportion of myristic acid and low level of oleic acid (Strong 1963, Barlow 1964, Fast 1964). Only trace amounts of fatty acids having carbon chain length more than 20 were found in B. germanica. This is in agreement with the observations of most workers. Giral (1946) and Giral, Giral and Giral (1946) reported 25.8% and 46.2% of fatty acids were more than 20 carbon chain long in S. purpurescens and M. atlanis respectively. Albrecht (1961) reported 72% stearic acid in Schistocerca gregaria Forsk. and is a very high value when compared with the iodine number. Usually stearic acid content in insects is lower than 10% (Fast 1964).

The loss in triglyceride is greater than dry matter loss during embryonic development. It is possible that part of the triglycerides are hydrolyzed to 1,2 diglyceride and utilized for the synthesis of phospholipids via phosphatidic acid. Part of the loss is accounted for by the increase in mono- and di-glyceride content during embryonic development (Kinsella and Smyth 1966, Gilbert 1967). The fatty acids released from the glycerides may be oxidized completely or utilized for the synthesis of sterol esters.

The phospholipid content increases during embryonic and post embryonic development. During incubation, the increase in the proportion of phospholipids has been shown to be partially due to synthesis and partially

due to utilization of triglycerides for energy requirements. Phospholipids are parts of cellular and subcellular membranes (Ansell 1964) and hence will increase during morphogenesis. Similar lipid patterns have been reported by Tichimirov (1885) in M. americana; by Pearincott (1960) in Musca domestica L.; Bieber et al. (1961) in Phormia regina (Meigen); Allais et al.(1964) in L. migratoria; Kinsella (1966a) in P. americana; and Gilbert (1967b) in L. maderae.

The relative proportion of the three major phospholipid classes lecithin, cephalin and sphingomyelin was quite similar to that reported by Allais et al.(1964) in L. migratoria and Siakotos & Zoller (1960) and Kinsella (1966) in P. americana. Many dipterans are characterised by the predominance of cephalin over lecithins (Fast 1964).

When calculated on a per individual basis, the lipid phosphorus content increased throughout development as a result of incorporation of non-lipid phosphorus into the phospholipid fraction. Chojnaki (1961) and Chojnaki and Piechowska (1961) studied the mechanism of synthesis in Celerio euphorbiae (Fab.) and found it to be similar to biosynthesis in vertebrate liver. Phosphocholine (or phosphoethanolamine) is activated by reaction with Cytidinetriphosphate to yield Cytidinediphosphate-choline (or ethanolamine) intermediate which then reacts with α,β diglyceride to yield choline (or ethanolamine)phosphatide.

The three roach species whose lipid metabolism during embryogenesis has been studied, have different oviposition habits. In P. americana the ootheca is extruded and carried by the female for only a short period, then deposited. In B. germanica ootheca is extruded and

carried by the female until the eggs hatch. In L. maderae the ootheca is extruded, then subsequently retracted into a brood sac until or shortly before hatching (Roth and Willis 1954). The incubation period of P. americana is twice as long as that of the other two species. The lipid metabolism pattern is, however, similar in all the three species. Thus oviposition or incubation period has very little effect on lipid metabolism.

The overall lipid pattern shown here is in agreement with Needham's theories concerning metabolism in terrestrial eggs. The greatest loss of lipids during the later stages of embryonic development is consistent with Needham's (1931) theory of a succession of energy sources in ontogeny. As pointed out by Needham (1931) increased fat catabolism, with suppression of protein breakdown, in cleidoic eggs is advantageous. This overcomes the difficulty of disposing of toxic end products of protein catabolism.

II.5. Summary:-

During the embryonic development of B. germanica there is an increase in the moisture content and a decrease in the dry matter and lipid content. The reduction in the lipid content was due to catabolism of triglycerides. The neutral lipid and triglyceride content decreased by 43% and 54% respectively. The decrease occurs mostly in the second half of development. On the other hand the mono- and di-glyceride content increased throughout incubation. Phospholipid content increased by 60% during embryogenesis. The major components of the phospholipid fraction are phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin. In addition phosphatidyl inositol, lysolecithin and phosphatidic acid were present in small amounts. Hydrocarbon and sterol content showed slight increase.

With the progress of embryonic development the proportion of esterified sterols increased. The overall decrease of 75% of the initial lipid store shows that lipids play a dominant role in fulfilling the energy requirements of developing eggs.

During nymphal development, the insect accumulates large amounts of lipid. Increase in the total lipid, neutral lipid and phospholipid content is proportional to the increase in the wet weight of the body. Though the adults had a lower fat content than the last instar nymphs, the lipid patterns are similar. In all the stages studied, triglycerides was the predominant fraction.

Fatty acid analysis of the phospholipid, neutral lipid and total lipid extracts revealed the presence of 17 fatty acids during all stages of the life cycle ranging in carbon chain length from 6 to 22. Palmitic, oleic and linoleic acids were the major fatty acids. Unsaturated fatty acids predominated in the various fract ons with oleic acid comprising about 45% of the total fatty acids in all the fractions studied. Palmitic acid was the second most abundant in the neutral lipid fraction, but linolenic was the second most abundant in the phospholipid fraction. Fatty acids of the phospholipids were more unsaturated than the fatty acids of the neutral lipids.

III. EFFECT OF DIETARY FATTY ACIDS ON GROWTH, DEVELOPMENT, MATURATION AND FATTY ACID COMPOSITION OF THE BODY FAT OF B. GERMANICA.

1. Introduction:-

Quality and quantity of the body fat of insects can be affected by their diets. The influence of dietary lipids on the composition of the body fat has been examined by numerous workers by comparing the Iodine Number of the dietary fat with that of the depot fat.

Yuill and Craig (1937) found that the larvae of Lucilia sericata (Meigen) reared on fish heads had depot fats with a high Iodine Number (140), which was similar to that of the fish. When reared on a diet containing butter, the depot fat had greater degree of saturation (Iodine Number 60). Mellampy and Maynard (1937) observed similar, though less conclusive, effects of the dietary fat on the depot fat of B. germanica.

Strong (1963a) obtained no indication that the host plant appreciably affected the fatty acid composition of Myzus persicae Sulzer and Macrosiphum pisi (Kaltenbach) and concluded that the fatty acid composition may be a characteristic of the species rather than a manifestation of the diet.

House, Riordan and Barlow (1958) observed that the degree of saturation of body lipids of Pseudosarcophaja affinis Fallen was directly influenced by the degree of saturation of dietary lipids. Barlow (1965) reared larvae of Agria affinis (Fallen) on chemically defined diets to which various fatty acids had been added. When reared on a fatty acid free diet the palmitoleic acid content of the larvae was high. Palmitoleic acid content also increased when oleic acid was omitted from the diet. The oleic concentration was reduced when no oleic acid was added to the diet and increased when high concentrations were fed. When a

mixture of fatty acids was added to the diet, there was an increase in the palmitic acid content and decrease in the palmitoleic acid content.

Barlow (1966) observed that the concentration of lipid in the body of the larvae of M. domestica was directly related to the fatty acid content in the diet. Lack of oleic acid in larval diet had the same effect as that of the absence of all fatty acids and produced a high proportion of palmitic, stearic and palmitoleic acids, and a low level of oleic acid in the body fat. Linoleic acid was absent from the body fat unless it was present in the diet.

Bumgarner and Lambremont (1966) analysed the fatty acid content of adult males, females and the eggs produced by these females reared on a diet with high fat content. They found a close resemblance between the dietary fatty acid and the fatty acid composition of body fat. When fed on a low fat diet, there was considerable difference in body fatty acids and fatty acid composition of the diet. They postulated that the females feeding on a low fat diet deposited lipids in the egg yolk which contain principally those fatty acids that the adults synthesized from non lipid components.

Brecken and Barlow (1967) found the ichneumonid parasitoid, Exeistes comstockii (Cress) to have no characteristic fatty acid composition but duplicated that of its host.

III.2. Materials and methods

III.2.1. Synthetic diet:-

The nymphs were reared on a chemically defined diet, under non-aseptic conditions. The composition of the basal diet was similar to the one formulated by Noland and Bauman (1949), except for the addition of 2 ppm of cyanocobalamin. The linseed oil in the diet was replaced by individual fatty acids (99.5% or greater purity). The composition (per 100 g of diet) was as follows:

Glucose	31.4 g	para-amino benzoic acid	30.0 mg
Casein	30.0 g	Nicotinic acid	10.0 mg
DL Methionine	1.8 mg	Thiamine HCl	7.0 mg
Non-nutritive fibre (NNF)	31.0 g	Ca pantothenate	4.0 mg
		Pyridoxine HCl	1.8 mg
Wesson salt mixture	4.0 g	Riboflavin	1.8 mg
Fatty acid	2.0 g	Folic acid	0.6 mg
Cholesterol	1.0 g	Biotin	0.6 mg
Choline chloride	0.4 g	Cyanocobalamin	0.2 mg
Inositol	0.04 g		

III.2.2 Preparation of the synthetic diet:-

In order to simplify diet preparation and ensure homogeneous mixing, a number of partial mixes were prepared and these were mixed in certain proportion as described below to give the desired level of a particular nutrient.

Mix N 1:- Glucose (31.4 g), casein (30.0 g), and Wesson salt mixture (4.0 g) were mixed and grounded into a fine powder with a mortar and pestle and roll mixed in a jar for one hour.

Mix N 2:- Water impregnate of cyanocobalamin (4 mg), nicotinic acid (400 mg), calcium pantothenate (80 mg), thiamine hydrochloride (140 mg), and pyridoxine hydrochloride (36 mg) in 100 g of non nutritive fibre (NNF) was prepared and vacuum dried. This was then finely ground in a ball-mill.

Mix N 3:- This contained 400 mg of inositol, 300 mg of p-amino benzoic acid, 18 mg of DL methionine, 18 mg of riboflavin in 20 g of NNF.

Mix N 4:- Sixty mg of folic acid were dissolved in hot dilute ammonia and impregnated in NNF to give 50 g.

Mix N 5:- Thirty mg of biotin was dissolved in hot 95% ethanol and impregnated into 50 g of NNF.

Mix N 6:- 800 mg of choline chloride were dissolved in water and impregnated in 10 g of NNF and dried in vacuum. This was then well grounded.

Cholesterol and fatty acids were added as the final step in diet preparation. Calculated amounts were dissolved in diethyl ether and were impregnated into each diet lot.

For making 100 g of diet, the following amounts of each mix were used:

Mix N 1	65.4 g
Mix N 2	5.0 g
Mix N 3	2.0 g
Mix N 4	0.5 g
Mix N 5	1.0 g
Mix N 6	5.0 g

To this 18.1 g of NNF was added and was well mixed in ball-mill. As mentioned earlier, cholesterol and the fatty acid were dissolved in ether and impregnated into this mixture.

Newly hatched nymphs were weighed and placed in the rearing jars. Food and water was changed every 5th day. Every 5th day the jars were inspected and the number of nymphs surviving and the weight of 10 nymphs were recorded. After the 50th day, the number and sex of the adults emerging were only recorded. After the emergence of the first adult, the jars were examined every day and the sex and number of adults emerging were noted down.

Growth index for each stage was calculated as follows:

$$\text{Growth index} = \frac{\text{Number of nymphs surviving} \times \text{mean wt. in mg/nymph}}{\text{Number of nymphs at 0 days} \times \text{mean wt. in mg/nymph at 0 days}}$$

Method of extraction, purification, preparation of methyl esters and GLC analysis of fatty acids has been described on pages 8 to 11 of this thesis.

III.3. Results

III.3.1. Growth survival and sex ratio:-

The effects of various fatty acids ranging in carbon chain lengths from 14 to 22 when added singly to the basal diet on the mean weight of the insects and their survival at various time intervals are shown in Table 14. From this the growth index was calculated and presented in Figure 5.

Figure 5 clearly shows that the addition of fatty acids more than 18 carbons long (with the exception of behenic acid) resulted in a larger growth index than the control diet. Addition of unsaturated 18 carbon acids, in particular, increased the growth rate. On the other hand,

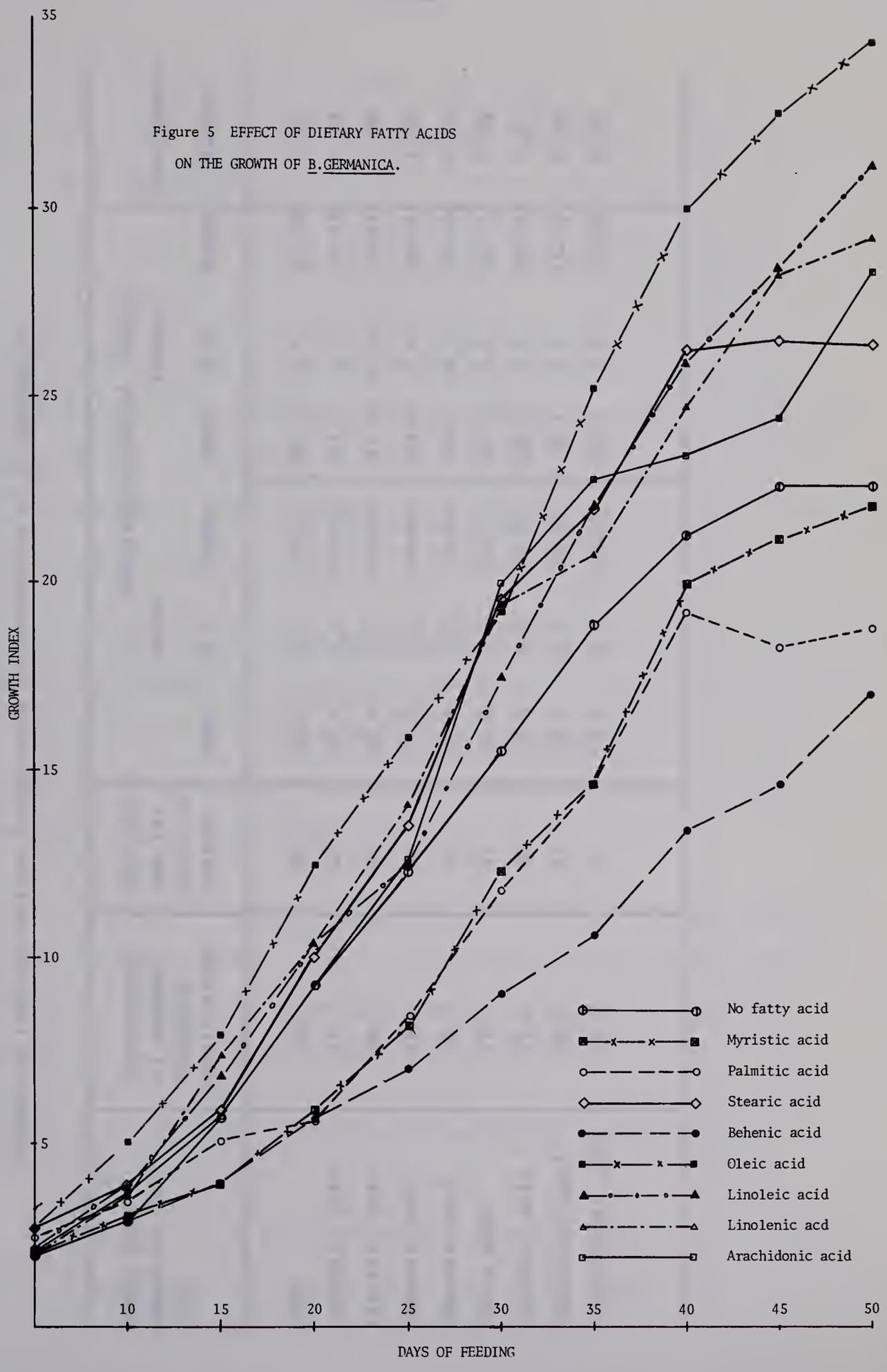


Figure 5 EFFECT OF DIETARY FATTY ACIDS ON THE GROWTH OF B.GERMANICA.

Table 7

Effect of feeding various fatty acids on maturation of *B. germanica*

Fatty acid added to the diet	Percentage of nymphs reaching adult stage	Days for the 1st adult to emerge	Maturation period (days) Males			Females			Male/Female ratio
			Mean	S.D.	Range	Mean	S.D.	Range	
None	52.6	45	52.2	4.5	45-62	54.4	4.4	45-64	0.81
Myristic acid	47.3	47	53.6	4.1	48-62	53.3	3.6	47-61	0.92
Palmitic acid	54.7	45	52.4	3.7	45-60	51.8	4.0	45-61	1.28
Stearic acid	47.9	46	52.8	3.2	46-60	53.0	3.4	48-61	1.05
Behenic acid	38.3	50	57.6	3.9	50-64	58.1	4.2	52-65	0.92
Oleic acid	57.7	41	47.4	2.7	41-53	47.9	2.6	42-55	0.94
Linoleic acid	57.0	40	47.4	3.4	40-56	48.0	2.5	40-56	0.92
Linolenic acid	56.7	41	48.9	4.1	41-56	48.1	3.9	41-57	0.89
Arachidonic acid	51.3	47	53.4	4.0	47-61	52.3	2.8	47-58	0.86

single addition of myristic, palmitic and behenic acids inhibited nymphal growth but did not affect survival. The highest growth index was recorded when nymphs were reared on a diet containing oleic acid.

The effect of the various diets on the survival to adult emergence and on sex ratio are shown in Table 7. This is based on pooled data of 5 tests. More nymphs reached the adult stage when the nymphs were reared on diets that contained palmitic, oleic, linoleic and linolenic acids as compared to the control diet. Addition of myristic, stearic and behenic acids, the last one in particular, to the diet resulted in fewer nymphs reaching maturity.

Addition of 18 carbon unsaturated fatty acids, (oleic, linoleic, and linolenic acids) also resulted in the nymphs reaching maturity earlier than either the control diet or diets containing saturated fatty acids. There was no difference between the mean maturation period of males and females in a single diet (Table 7).

Inclusion of fatty acids in the diet tended to bring the ratio of adults obtained nearer to unity. The proportion of males was higher when palmitic and stearic acids were added to the diet. In all the other instances, the females were preponderant.

III.3.2. Effect of dietary fatty acids on lipid content and fatty acid composition:-

Tables 8 and 9 show an increase in the % lipid on a wet weight basis in B. germanica adults when fatty acids were added to the control diet. Lipid content was highest when a fatty acid mixture was fed. Addition of di- and tri-ethanoid 18 carbon fatty acids to the diet resulted in greater lipid

Table 8

Effect of dietary fatty acid on the fatty acid composition of body fats of adult male B. germanica

Fatty acid added to the diet	Lipid Content % of wet wt.	% fatty acid composition						
		$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ & $C_{20:0}$
None	2.35	0.9	23.6	6.9	4.3	58.7	5.0	0.6
Myristic acid	3.21	5.3	28.0	6.6	3.8	51.1	4.7	0.5
Palmitic acid	2.98	0.9	33.0	7.9	3.5	49.1	4.6	0.5
Stearic acid	3.06	1.0	27.4	7.0	5.0	55.6	3.6	0.4
Oleic acid	3.65	0.7	25.7	4.0	3.9	60.8	3.9	1.0
Linoleic acid	3.46	1.0	28.4	5.1	3.1	45.9	15.4	1.1
Linolenic acid	3.98	1.0	26.5	4.8	3.2	43.4	16.1	5.0
Fatty acid mixture*	4.04	0.9	24.5	5.0	3.6	48.5	14.3	3.2

*Fatty acid mixture contained equal parts of myristic, palmitic, stearic, oleic, linoleic and linolenic acids. Fatty acids added 2% by wt. $C_{14:0}$ Myristic acid; $C_{16:0}$ Palmitic acid; $C_{16:1}$ Palmitoleic acid; $C_{18:0}$ Stearic acid; $C_{18:1}$ Oleic acid; $C_{18:2}$ Linoleic acid; $C_{18:3}$ Linolenic acid; $C_{20:0}$ Arachidic acid.

Table 9

Effect of dietary fatty acid on the fatty acid composition of B. germanica adult females

Fatty acid added to the diet	Lipid Content % of wet wt.	% fatty acid composition						
		$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ & $C_{20:0}$
None	3.02	0.6	20.4	7.6	4.1	60.7	5.9	0.7
Myristic acid	4.61	8.7	27.9	9.9	2.9	47.1	3.5	—
Palmitic acid	3.69	1.1	35.9	10.3	2.8	48.5	1.4	—
Stearic acid	4.43	0.6	26.7	6.6	5.7	58.5	1.2	0.7
Oleic acid	4.94	0.8	28.9	3.7	4.3	60.4	1.2	0.7
Linoleic acid	5.20	0.7	27.7	5.2	3.4	48.3	14.1	0.6
Linolenic acid	5.08	0.9	24.8	4.1	3.3	45.1	15.7	6.1
Fatty acid mixture*	5.36	1.4	17.5	3.8	5.6	55.2	12.8	3.7

*Fatty acid mixture contained equal parts of myristic, palmitic, stearic, oleic, linoleic and linolenic acids.

$C_{14:0}$ Myristic acid; $C_{16:0}$ Palmitic acid; $C_{16:1}$ Palmitoleic acid; $C_{18:0}$ Stearic acid; $C_{18:1}$ Oleic acid; $C_{18:2}$ Linoleic acid; $C_{18:3}$ Linolenic acid; $C_{20:0}$ Arachidic acid.

content than in those reared on diets to which saturated fatty acids had been added.

The fatty acid content in Tables 8 and 9 are expressed as % of the total fatty acid content. A lower proportion of a certain fatty acid in adults obtained from one diet does not necessarily mean that the fatty acid was less in absolute amounts per individual.

The fatty acid composition of the total fat shows interesting trends. Lack of polyunsaturated fatty acids in the diet resulted in a large reduction of linoleic and linolenic acid content. For example, the concentration of linoleic acid in the adults emerging from the control diet and other fatty acid diets was about an eighth that of the adults emerging from a diet with linoleic acid. Similarly, the linolenic acid content increased from 0.6% to more than 5.0% when this fatty acid was fed. Addition of any fatty acid to the diet fed produced an increase in the proportion of that particular fatty acid in the body fat. The increase seems to be related to the amount fed. Oleic acid was the most abundant fatty acid in body fat of adults emerging from all the diets tested.

III.4. Discussion

Results of the nutritional studies indicate that, although dietary fatty acids are not essential for growth of B. germanica addition of unsaturated fatty acids accelerates nymphal growth and increases the percentage of adults obtained. This work confirms an early report (Gordon 1959) that addition of oleic, linoleic or linolenic acid to the diet promotes faster growth and increases the number of adults obtained. B. germanica, however, needed linoleic acid for reproduction, and deficiency symptoms became manifest in the progeny of deprived parents (Gordon 1959).

Few insects have been found to need a dietary source of lipids other than cholesterol. Fats have been found essential for the moths of the genus Ephestia (Fraenkel and Blewett 1946), Pectinophora gossypiella (Saund.) (Beckman, Bruckart and Reiser 1953) and L. migratoria (Dadd, 1960, 1961). Other works have shown that addition of unsaturated fatty acids promotes growth (House and Barlow 1960, Vanderzant, Kerur and Reiser 1957).

The presence of lipids in the German roaches reared on fat free diet indicates that they have the ability to synthesize lipids from non-lipid materials. It is well known that insects have the ability to synthesize lipids from carbohydrates and proteins. Clements (1959) found partial incorporation of carbon atoms of amino acids into the fat by locust fat body. Synthesis of fats has also been reported from C^{14}-glucose (Zebe and McShan 1959, Van Handel and Lum 1961, Strong 1963). Synthesis of lipids from acetate, an important intermediate of carbohydrate metabolism has been demonstrated in many insects. In vitro studies have shown that incorporation of ^{14}C-acetate into the lipids occurs in S. gregaria (Clements 1959), Prodenia eridania (Cramer) (Zebe and McShan 1959), and L. migratoria (Tietz 1961).

In in vivo studies also, acetate was found to be incorporated into the lipids of Dermestis maculatus De Geer (Bloch et al.1956), P. americana (Louloudes et al. 1961), Musca domestica L. (Robbins et al. 1961), Calliphora vicina Robineau-Desvoidy (Sedee 1961), Corcyra cephalonica Stainton (Rajalakshmi, Sarma, and Sarma 1963), Myzus persicae (Sulzer) (Strong 1963b), Eurycotis floridana Walk. (Bade 1964), Bombyx mori L. (Stridhara and Bhat 1964, 1965a, 1965b), Hyalophora cecropia L. (Chino and Gilbert 1965), and Anthonomus grandis (Boheman) (Lambremont 1965, Lambremont, Stein and Bennett 1965). Synthesis of fatty acids occurs

by the condensation of acetyl CoA to malonyl CoA, followed by the liberation of carbon dioxide and the formation of ketoacyl-CoA which can then react further with malonyl CoA. Repetition of this process builds up a complete chain of fatty acids and the keto groups are then reduced with NADPH (Gilmour 1965, Gilby 1965).

In the present work small amounts of linoleic and linolenic acids were detected in roaches that were reared on diets containing no source of these acids. In this connection it is interesting to note that only very low order of incorporation of radioactivity occurs into linoleic and linolenic acids in most of the insects studied so far (Bloch et al.1956, Van Handel and Lum 1961, Bade 1964). On the other hand, Sedee (1961) and Louloudes et al.(1961) demonstrated synthesis of higher amounts of linoleic and linolenic acids, under non-aseptic conditions in C. vicina and P. americana respectively. Since the present work was also conducted under non-aseptic conditions, it is possible that inter and intracellular symbionts are responsible for the synthesis of these two polyunsaturated fatty acids.

III.5. Summary:-

Newly hatched nymphs were reared on artificial diets to which pure fatty acids had been added singly. Growth indices revealed that, although dietary fatty acids were not essential, addition of linoleic and linolenic acids promoted faster growth and increased the number of adults obtained. Addition of unsaturated fatty acids resulted in an increase in the body lipid content. Studies on the fatty acid composition of the adults obtained from these diets revealed that B. germanica or its symbionts can synthesize saturated fatty acids ranging in carbon chain length from

C_8 to C_{18}. Small amounts of polyunsaturated fatty acids, namely linoleic and linolenic acids, were also present in the body fat, possibly synthesized by the roach and/or symbionts. Addition of fatty acids at 2% level in the diet results in an increase in the proportion of the particular fatty acid added.

IV. TOXICOLOGICAL STUDIES

1. Introduction:-

Many workers have investigated the effect of lipid content and characteristics on the toxicity of insecticides. There appears to be a correlation between increased lipid content and unsaturation of fatty acids and tolerance of chlorinated hydrocarbon insecticides (Berim and Edelman, 1949; McGovern, 1949; Mer and Furmanska, 1953; Munson, 1953a,b; Munson and Gottlieb, 1953; Munson, Padilla and Weisman, 1954; Lofgren and Cutkomp, 1956; Fisk, 1958; Bridges and Cox, 1959; and many others).

Reiser *et al.*(1953) found that the adults of *A. grandis* surviving EPN (Ethyl p-nitrophenyl benzene thiophosphonate) and methyl parathion treatment at the rate of 0.30 and 0.15 pounds per acre had a higher fat content than those that died. Saito (1960) also suggested that susceptibility differences in *P. americana* to schradan could be explained on the basis of fat content. The experiment herein was conducted to obtain information regarding the effect of dietary fatty acids on toxicity of malathion to *B. germanica* adults.

IV. 2. Materials and Methods:-

Newly hatched nymphs were reared in battery jars on a fat free synthetic diet (composition given on page 35) to which pure fatty acids had been added singly or in combination. The temperature cabinet was maintained at 30 + 1 C. Emerging adults were collected every third day and kept in battery jars and maintained on the same diet on which they were reared as nymphs. Two to three week old adults were used for toxicological studies.

Malathion (0,0-dimethyl S-(1,2-dicarboethoxyethyl)phosphorodithioate) solutions in acetone were applied topically to weighed insects with a ISCO microapplicator fitted with a 27 gauge hypodermic needle. Carbon dioxide was used as an anaesthetic to facilitate handling of roaches while applying insecticide. The adults were anaesthetized and placed in a row on a small glass plate. The microapplicator was adjusted to deliver a desired volume and the tip of the needle with the insecticide solution was brought into contact with the metathoracic sterna of the insect. Different doses were obtained by varying the concentration of malathion in acetone. Generally a concentrated solution was prepared and diluted step by step with acetone to selected concentrations. After application of insecticide, the treated roaches were placed in glass tubes (9" X 1/2") and were placed in a temperature chamber maintained at 30 C.

Six levels of toxicant were used. Each level was replicated three times and 20 adults of each sex were used for each replicate. Mortality data were recorded 24 hours after treatment. The mortality data were subjected to probit analysis as described by Finney (1947).

IV. 3. Results :-

The number of adults dead 24 hours after treatment are shown in Tables 14 and 15. These were plotted on log probability papers and LD_{50} (µg of toxicant required to kill 50% of insects) values calculated according to Finney (1947). The calculated regression lines have been shown in figures 6a-h. The regression equation and LD_{50} values for males and females are given in Tables 10 and 11.

Table 10

Effect of dietary fatty acids on susceptibility of adult males of B. germanica to malathion

Fatty acid added to the diet	Lipid Content % of wet weight	Heterogeneity (degree of freedom 4)	Regression equation	LD_{50} µg/g of body wt.	Relative Susceptibility
No fatty acid	2.35	$X^2 = 0.986$	Y = 3.7723 x -1.8820	66.3	1.00
Myristic acid	3.21	$X^2 = 3.280$	Y = 3.110 x -1.0037	85.4	0.78
Palmitic acid	2.98	$X^2 = 1.167$	Y = 3.8725 x -2.4885	90.5	0.73
Stearic acid	3.06	$X^2 = 2.861$	Y = 3.6894 x -2.3384	97.5	0.68
Oleic acid	3.65	$X^2 = 4.198$	Y = 3.2074 x -1.4326	101.3	0.65
Linoleic acid	3.46	$X^2 = 2.292$	Y = 3.4522 x -1.6862	86.5	0.77
Linolenic acid	3.98	$X^2 = 3.986$	Y = 3.6448 x -2.0337	83.5	0.79
Fatty acid mixture	4.04	$X^2 = 6.973$	Y = 3.8760 x -2.5764	90.1	0.74

Y = Probit kill X = µg of malathion/g of body weight LD_{50} median lethal dose

Table 11

Effect of dietary fatty acids on susceptibility of adult females of B. germanica to malathion

Fatty acid added to the diet	Lipid content % of wet weight	Heterogeneity (Degrees of freedom 4)	Regression equation	LD_{50} µg/gm of body wt.	Relative Susceptibility
No fatty acid	3.02	$X^2 = 1.980$	Y = 3.6259 x -1.5295	61.3	1.00
Myristic acid	4.61	$X^2 = 0.955$	Y = 2.9814 x -0.7659	85.9	0.72
Palmitic acid	3.69	$X^2 = 2.757$	Y = 3.2276 x -1.4455	99.3	0.62
Stearic acid	4.43	$X^2 = 2.067$	Y = 3.4372 x -2.1047	116.7	0.53
Oleic acid	4.94	$X^2 = 8.235$	Y = 3.7242 x -2.3238	92.6	0.66
Linoleic acid	5.20	$X^2 = 0.939$	Y = 3.5491 x -2.0236	95.4	0.64
Linolenic acid	5.08	$X^2 = 0.704$	Y = 3.6726 x -2.0438	82.8	0.74
Fatty acid mixture	5.36	$X^2 = 2.174$	Y = 3.7731 x -2.4336	93.3	0.66

Y = Probit kill X = µg of malathion/g of body weight LD_{50} median lethal dose

For males the dietary effects on susceptibility based on median lethal dose (μg/g body wt) in the descending order (most susceptible to least susceptible) was control, lonolenic, myristic, linoleic, palmitic, fatty acid mixture, stearic and oleic acid. In the females the order was as follows: control, linolenic, myristic, oleic, linoleic, fatty acid mixture, palmitic and stearic acid.

The ratio of the highest LD_{50} values to lowest was about 1.5 and 2.0 in males and females respectively.

IV.4. Discussion:-

A comparison of the total lipid content and the LD_{50} values for the various treatments reveal that there was no direct relationship. For example adult males obtained from the fatty acid mixture diet have a lower LD_{50} value than those from any other diet except the control. The fat content, however, was highest. Adding myristic acid to the nymphal diet resulted in a greater susceptibility to malathion than addition of palmitic and stearic acids. The lipid content of the adults, however, was higher than those from the myristic acid diet.

Similarly, adult females from a diet containing a fatty acid mixture were more susceptible to malathion than females from diets containing palmitic, stearic, oleic and linoleic acids. Fat content, on the other hand, was highest in females from the fatty acid mixture diet. Adults from palmitic acid diet were more tolerant than adults from myristic acid, oleic acid and linoleic acid diets though it had a lower fat content. Adults from a linolenic acid diet were more susceptible than adults from diets containing myristic, oleic and palmitic acids. Fat content in

adults emerging from the linolenic acid diet was higher than the other diets mentioned above.

Females obtained from nymphs reared on diets containing oleic, linoleic, linolenic and fatty acid mixture had 70% unsaturated fatty acids in their body fat. Adult females emerging from the control diet were more susceptible than the adults from the diets containing myristic and palmitic acids. Addition of palmitic and myristic acids increased the degree of saturation of the body fats. Thus it could be concluded that there was a relation between the degree of saturation (or unsaturation) of the body fats or the total fat content and susceptibility to malathion. Fast (1964) suggested the possibility of change in vigor as a result of dietary changes as the cause of difference in tolerance.

IV.5. Summary:-

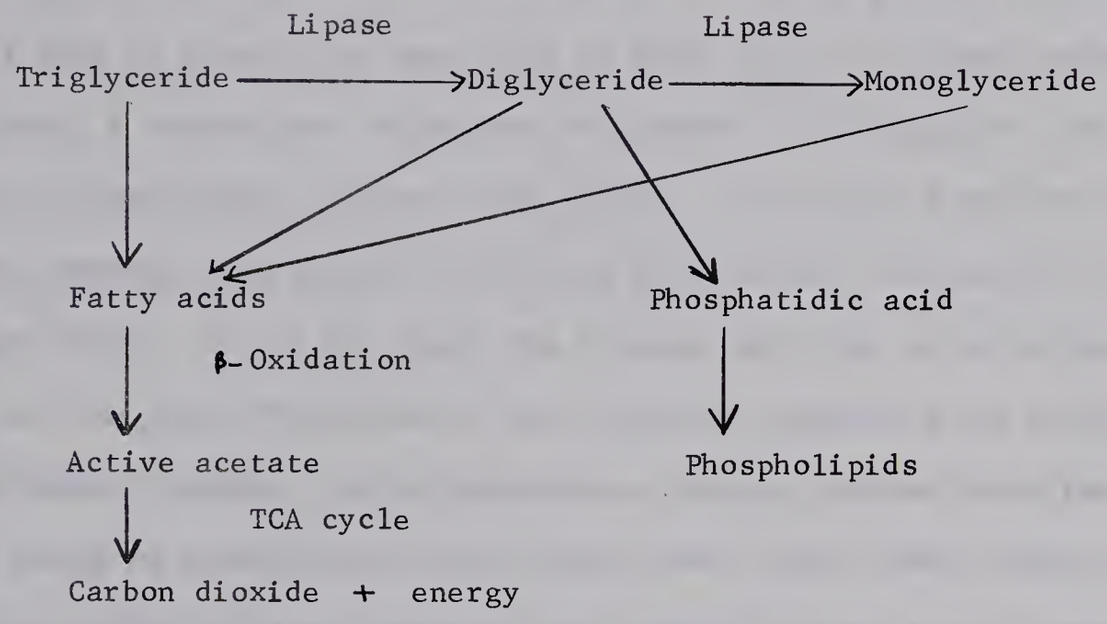
B. germanica nymphs were reared on artificial diets to which various fatty acids had been added. Adults emerging from these were tested for their susceptibility to malathion applied topically. Addition of fatty acids to the diet increased the LD_{50} values in both sexes. Susceptibility, however, is not related to either total lipid content or degree of saturation of the body fats.

V. GENERAL DISCUSSION AND CONCLUSIONS

The changes in the lipid composition of B. germanica during embryonic and post embryonic development was studied using a combination of column, thin-layer and gas-chromatographic techniques.

During embryonic development, the wet weight of the eggs increases due to absorption of water from the female, and the dry matter content decreases. The loss of dry matter has been shown to be primarily due to utilization of triglyceride fatty acid moiety of triglyceride fraction for energy requirements. The fatty acids released are split into 2 carbon units, "active acetate", which can be totally oxidized via TCA cycle. It is interesting to note that the loss in triglyceride fraction is greater than the loss of the neutral lipid fraction. It is possible that triglycerides are only partially hydrolyzed (either one or two of the three fatty acid moieties removed). This is supported by the fact that there is an increase in the mono- and di-glyceride content. In addition, part of the di-glycerides formed may be utilized for synthesis of phospholipids via phosphatidic acid.

The overall triglyceride metabolism during embryogenesis can be summarized as follows:



Loss of lipids accounted for 75% of the loss of dry matter during embryogenesis. This shows that lipids form the major energy substrate and is in conformity with Needham's first hypothesis (1931) that lipid catabolism is characteristic of terrestrial eggs. Though lipids are the main source of energy, substances other than lipids, particularly carbohydrates, may have been used for energy requirements. The greatest decrease of lipids occurred during the later stages of embryonic development and is in accordance with the generalization postulated by Needham (1931) regarding succession of energy sources during ontogeny.

During nymphal development, all the lipid fractions increase. In order to compare the rate of increase of lipids with that of wet weight, log of wet weight and log of total lipids of the different nymphal instars were plotted and a straight line was drawn. The total amount of fat was related to the wet weight by the equation $y = b\ x^k$, where k is known as the

heterauxetic constant. The same formula may also be written in the logarithmic form $\log y = \log b + k \log x$. The value of k was close to 1 indicating that the rate of accumulation of fat during nymphal growth occurs more or less at the same rate as that of the total body weight. Similarly, k values were calculated for neutral lipids against total lipids, phospholipids against total lipids, triglycerides against neutral lipids, sterols with neutral lipids and hydrocarbons with neutral lipids (Figure 7a-h). In all the cases the k value was close to unity indicating that all the rate of increase of each fraction paralleled the increase in the other fraction. In holometabolous insects, on the other hand, the k value is greater than unity (Finkel 1948, Fast 1964) indicating that the weight of lipid increases more rapidly during larval growth than the total weight. Accumulation of large amount of fat is advantageous for holometabolous insects, because considerable energy is required for transformation into adult. In addition many adults do not feed and depend upon fat reserves.

The fatty acid composition of all the nymphal instars and the adults are quite similar. These results may be interpreted to mean that there is no selective synthesis or accumulation of any fatty acids during nymphal growth.

Nutritional studies revealed that fatty acids are not required for growth. However, addition of unsaturated fatty acids resulted in a faster growth rate and a greater number of nymphs reached the adult stage. It has also been shown that adults obtained from a diet without any fatty acid, contained fatty acids including small amounts of linoleic acid and linolenic acid. This shows that B. germanica L. or its symbionts can

synthesize fatty acids from non lipid precursors, and to a great extent fulfill its fatty acid requirements. Addition of linolenic or linoleic acid increased the proportion of these acids in the body fat. The limited amount of linoleic and linolenic acid found in adults obtained from diets to which these fatty acids had not been added may be (i) synthesized by symbionts (ii) the roaches have the ability to synthesize them in very small amounts or (iii) may be due to a carry-over from the embryonic reserves.

It must be emphasized that the nutritional studies were conducted on a single generation of B. germanica and the results indicate requirements for a single generation. Nutritional studies on two or more consecutive generations may reveal additional requirements (Gordon, 1959).

The present study leads to a number of lines of work; how far symbionts are responsible for synthesis of polyunsaturated fatty acids. It is worthwhile studying synthesis of fatty acids from C^{14} acetate in aposymbiotic roaches under aseptic conditions. If the depot fat can be changed by dietary means, this can be used for studying the possible relationship between lipids and cold hardiness. In B. germanica L. choline phosphatide predominates over ethanolamine phosphatide. In Diptera ethanolamine phosphatide predominates over choline phosphatide (Fast 1964). It would be interesting to study the effect of partially replacing the dietary choline, on the phospholipid composition of B. germanica L.

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*Reference seen as Abstract.

VII. APPENDICES

Table 12

Wet weight, dry weight and water content at the various stages of embryonic and post embryonic development of B. germanica

Stage of Development	Wet weight mg	Dry weight mg	Water content %
Ootheca			
0 days	38.29±0.29	14.58±0.25	61.93±0.38
5 days	37.55±0.26	14.29±0.29	61.94±0.31
10 days	43.25±0.27	13.60±0.17	68.55±0.47
15 days	48.50±0.16	11.98±0.27	75.30±1.35
Nymphs			
1st instar	2.25±0.10	0.62±0.07	72.58±0.85
2nd instar	5.09±0.14	1.49±0.07	71.31±1.86
3rd instar	9.92±0.16	2.99±0.09	69.86±0.89
4th instar	20.47±1.42	6.51±0.37	68.19±1.56
5th instar	36.85±1.38	11.59±0.29	68.52±1.21
6th instar	53.48±2.45	16.93±0.94	68.54±1.52
Adults			
Males	47.72±1.14	15.54±0.36	67.41±1.13
Females	64.78±0.98	19.79±1.51	69.44±2.46

Table 13

Proportion of various lipid classes in the neutral lipid fraction of the German cockroach (% of neutral lipid fraction)

Stage	Hydrocarbons	Sterol esters	Triglycerides	Sterols	Diglyceride	Monoglyceride	Free fatty acid
Ootheca							
0 day	1.16	0.89	94.35	2.49	0.24	0.55	0.32
5 day	1.27	1.05	91.84	2.46	1.00	1.52	0.86
10 day	1.62	1.44	88.51	2.17	1.83	2.31	2.12
15 day	2.56	3.21	75.87	3.49	4.29	5.63	4.94
Nymphs							
Instar 1	5.90	6.06	70.12	5.33	4.53	4.17	3.89
Instar 2	5.45	5.33	71.21	5.59	4.64	4.04	3.74
Instar 3	5.26	5.64	70.09	5.93	5.63	4.52	2.93
Instar 4	4.97	6.03	69.40	5.84	5.71	4.95	3.10
Instar 5	7.85	5.78	69.77	5.07	2.83	4.64	4.06
Instar 6	5.18	4.40	69.88	7.39	4.52	4.79	3.84
Adults							
Male	6.35	5.70	66.71	7.02	4.97	4.88	4.36
Female	6.40	6.41	71.94	4.48	4.42	3.90	2.45

Table 14

Growth and survival of B. germanica when various fatty acids were added singly in comparison with the control diet

Fatty acid added to basal diet	Mean weight (mg/nymph) and per cent survival (in parentheses) at time interval (days) after hatching									
	5	10	15	20	25	30	35	40	45	50
None added	2.64[a] (90.7)[b]	3.85 (81.3)	7.64 (79.3)	13.61 (76.7)	18.84 (73.3)	25.61 (68.0)	33.64 (63.3)	40.63 (58.7)	45.63 (55.6)	47.63 (54.6)
Myristic acid	2.72 (85.3)	4.51 (77.3)	6.67 (68.0)	10.54 (63.3)	15.26 (60.7)	23.90 (59.3)	28.84 (58.0)	39.55 (58.0)	42.80 (56.7)	45.0 (56.7)
Palmitic acid	3.35 (99.3)	4.97 (90.0)	7.55 (85.3)	10.46 (73.3)	16.06 (69.3)	24.00 (65.3)	31.69 (62.7)	40.37 (60.0)	43.30 (57.3)	46.9 (57.3)
Stearic acid	3.27 (87.6)	4.98 (78.7)	8.93 (72.8)	15.65 (69.7)	22.34 (65.3)	34.20 (62.7)	39.43 (60.6)	49.59 (57.9)	51.58 (56.2)	52.6 (55.0)
Behenic acid	2.62 (89.2)	4.92 (69.2)	7.55 (62.5)	10.99 (62.5)	14.61 (57.5)	18.46 (57.5)	23.56 (54.2)	30.04 (54.2)	35.43 (50.0)	41.5 (50.0)
Oleic acid	2.85 (91.6)	5.84 (86.5)	9.84 (80.6)	16.08 (77.7)	21.65 (72.6)	28.43 (68.1)	38.60 (65.8)	42.60 (62.2)	45.65 (62.6)	50.6 (62.6)
Linoleic acid	2.44 (97.0)	5.04 (82.6)	9.86 (77.7)	15.04 (74.1)	19.00 (68.0)	27.64 (64.5)	38.88 (62.5)	45.24 (61.5)	49.85 (61.5)	54.3 (61.5)
Linolenic acid	2.28 (98.0)	4.55 (90.0)	9.15 (78.6)	15.09 (76.0)	20.98 (74.0)	29.46 (69.3)	35.96 (64.6)	44.65 (64.0)	48.63 (64.0)	50.6 (64.4)
Arachidonic acid	2.68 (90.0)	5.08 (80.6)	9.00 (71.9)	14.68 (71.3)	22.69 (68.8)	33.65 (67.5)	39.45 (65.6)	43.61 (61.3)	48.95 (58.8)	53.6 (57.8)

a - mean wt/nymph in mg. b - % nymphs surviving.

Table 15

Number of B. germanica L. adult females dead 24 hours after topical application of malathion (60 insects/treatment)

Toxicant µg/g	Adult obtained from diet containing							
	No fatty acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Fatty acid mixture
303.75	60	59	60	57	60	59	59	59
202.5	58	53	54	44	56	52	55	54
135	48	39	40	39	38	42	47	44
90	39	30	28	18	24	31	35	28
60	25	21	15	10	19	15	16	15
40	12	9	8	3	6	6	8	7
Control	0	0	1	0	0	0	0	2

Table 16

Number of B. germanica L. adult males dead 24 hours after topical application of malathion (Number of insects per treatment-60)

Toxicant µg/g	Adult obtained from diet containing							
	No fatty acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Fatty acid mixture
303.75	60	60	59	60	58	60	60	60
202.5	58	51	56	54	47	54	56	54
135	51	41	43	41	39	44	44	46
90	41	29	30	29	29	30	28	30
60	27	19	14	13	13	19	18	10
40	13	10	6	8	8	9	9	7
Control	1	0	0	0	2	0	0	0

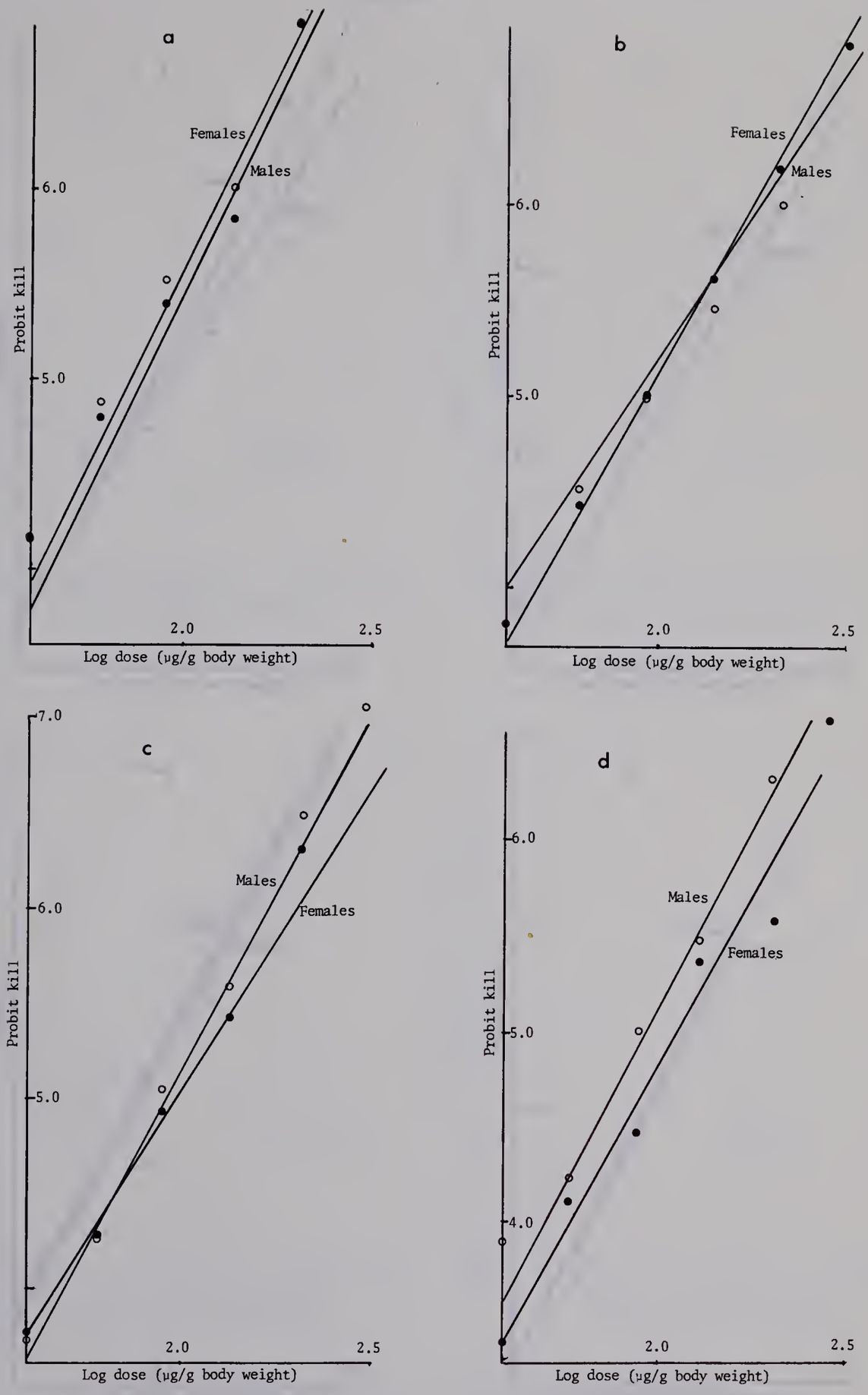

Figure 6 PROBIT REGRESSION LINES FOR TOXICITY OF MALATHION TO ADULTS OF B.GERMANICA REARED ON A DIET WITH (a) NO FATTY ACID; (b) 2% MYRISTIC ACID; (c) 2% PALMITIC ACID and (d) 2% STEARIC ACID.

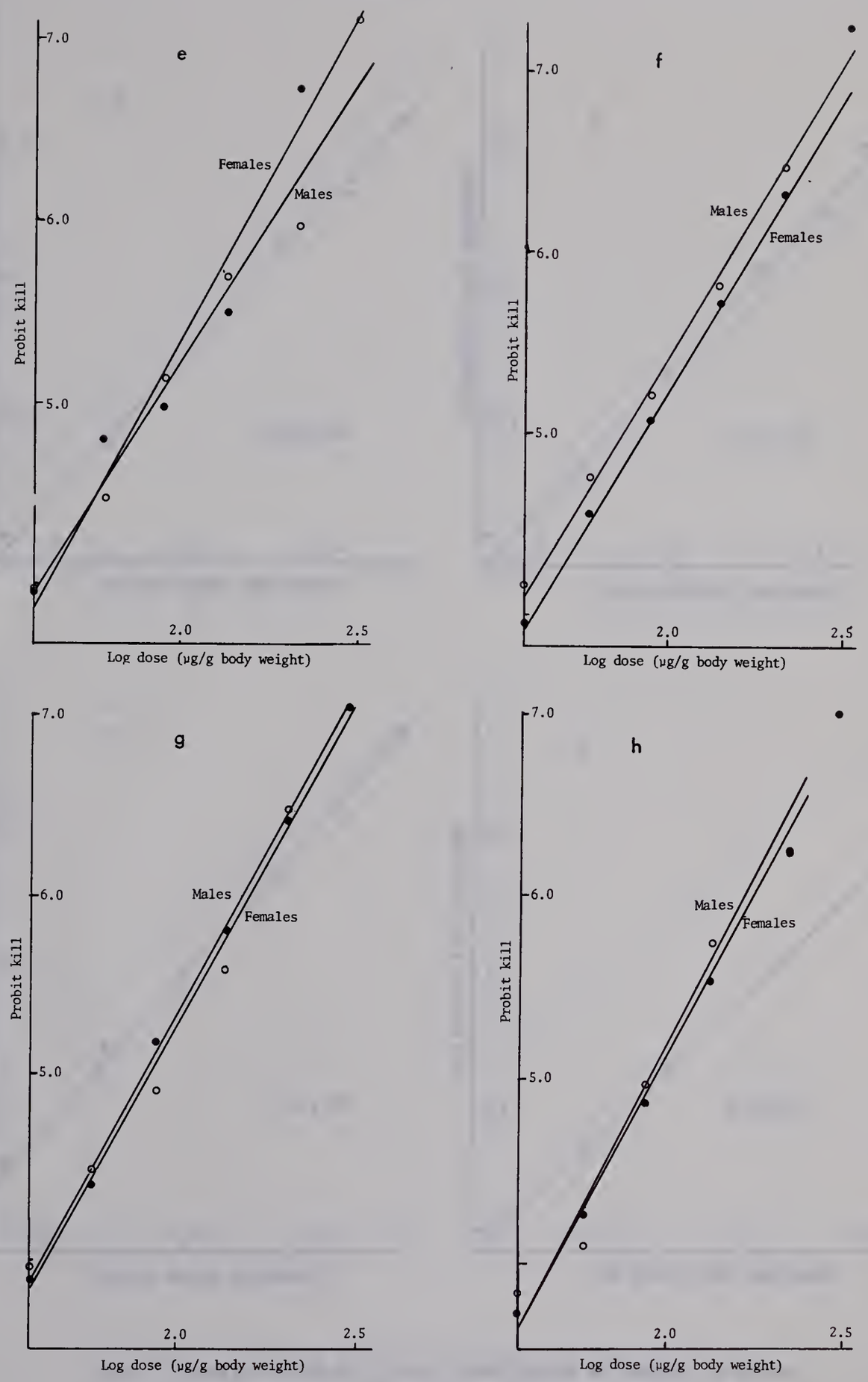

Figure 6 PROBIT REGRESSION LINES FOR TOXICITY OF MALATHION TO ADULTS OF B.GERMANICA REARED ON A DIET WITH (e) 2% OLEIC ACID; (f) 2% LINOLEIC ACID; (g) 2% LINOLENIC ACID; (h) 2% FATTY ACID MIXTURE.

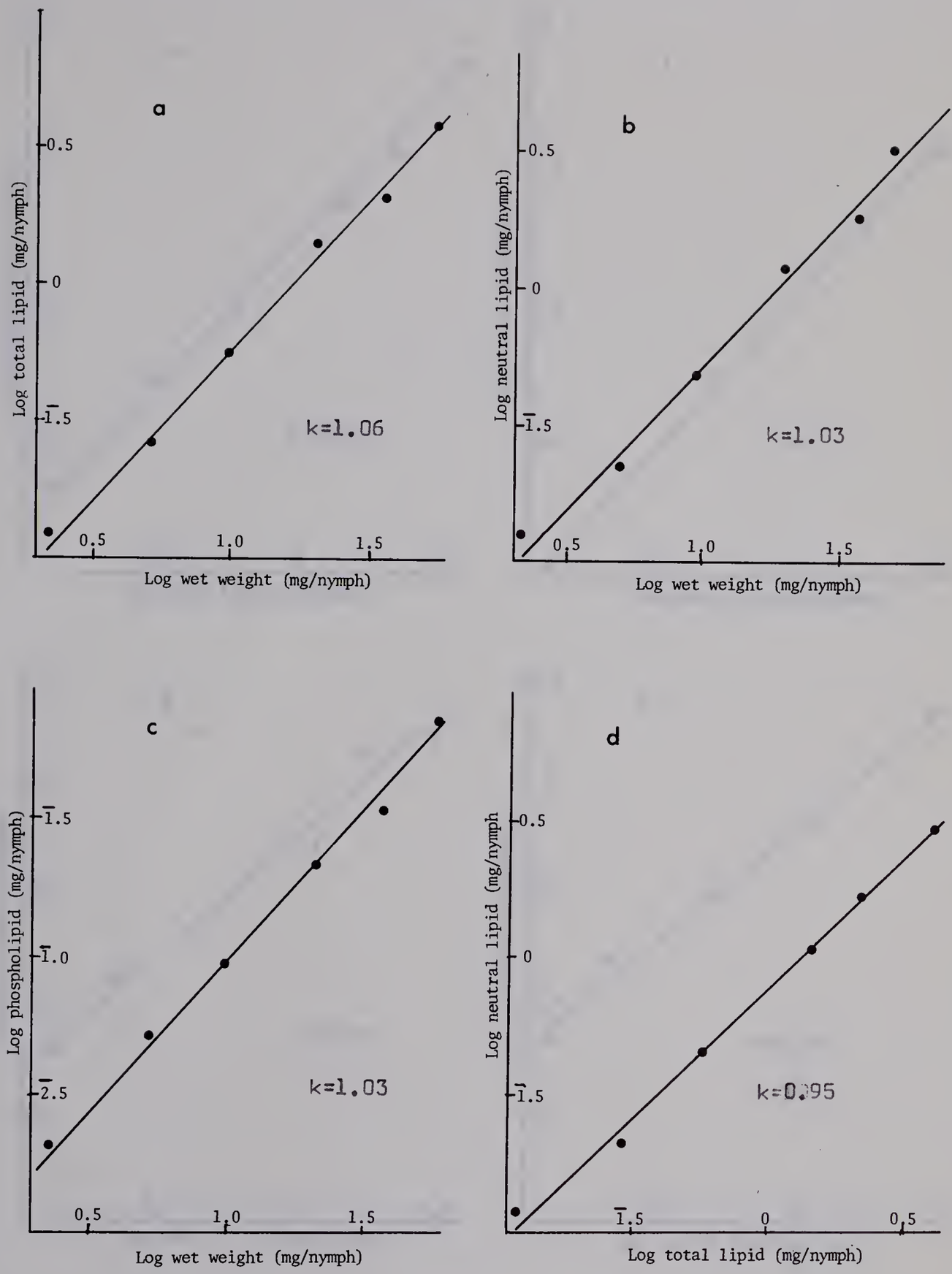

Figure 7. DOUBLE-LOG PLOT OF (a) TOTAL LIPID AGAINST WET WEIGHT; (b) NEUTRAL LIPID AGAINST WET WEIGHT; (c) PHOSPHOLIPID AGAINST WET WEIGHT and (d) NEUTRAL LIPID AGAINST TOTAL LIPID.

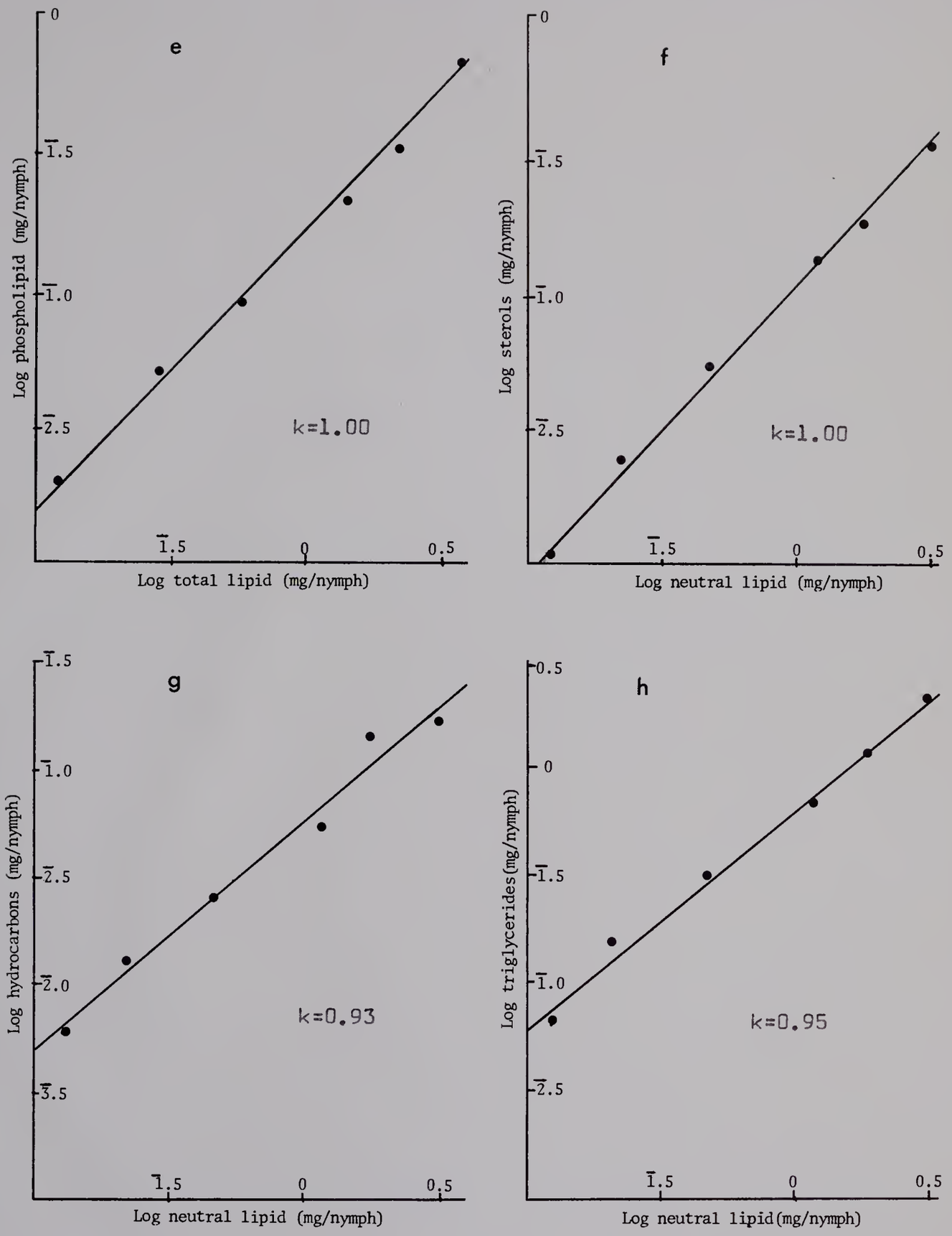

Figure 7. DOUBLE-LOG PLOT OF (e) PHOSPHOLIPID AGAINST TOTAL LIPID; (f) STEROLS AGAINST NEUTRAL LIPID; (g) HYDROCARBONS AGAINST NEUTRAL LIPID and (h) TRIGLYCERIDES AGAINST NEUTRAL LIPID.